PHOTOREACTIVATION

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Awake! for Morning in the Bowl of Night
Hath flung the Stone that puts the Stars to Flight
And lo! the *Streptomyces griseus*
Hath been Reactivated by the Light!

—Anon.

I. INTRODUCTION

Photoreactivation is the reversal with near-ultraviolet or visible light of ultraviolet radiation damage to a biological system. It usually involves the complete cancellation with 3-ev photons of a large fraction of the observable damage caused by 5-ev photons. This phenomenon is of great importance to radiobiology, for it may be a quite specific process and there is hope that its mechanism can be fathomed. Knowledge of this mechanism would be a long step toward an understanding of the biological actions of ultraviolet light.

This same phenomenon is also called "photorestoration," "photoreversal," or "photorecovery," and present knowledge does not indicate that any of these terms is superior to another. Latarjet and Gray (108) classed treatments that modify radiation response as "restorations" if they are started after irradiation begins. In addition, the term "restoration" has come to be applied to most posttreatments (*e.g.*, "catalase restoration"), and "photo*restoration*" would therefore be a term more consistent with existing nomenclature. However, because the term

[1] Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

"photo*reactivation*" has been used predominantly in the literature, is the title of the only previous review of the subject, and has been applied to several related terms (*e.g.*, "photoreactivable sector"), it appears to be too deeply intrenched to be changed now without creating confusion, and therefore this term is retained in the present article.[2]

The subject of photoreactivation through 1952 was reviewed by Dulbecco (40).[3] Since then, the volume of literature has roughly quadrupled, and a great deal more is now known about photoreactivation behavior. Although the basic picture of the process remains essentially the same, proposed mechanisms may now be examined more critically. In this article, I shall report on research between 1952 and 1958 and discuss current ideas on the mechanism of photoreactivation.

The review is planned in a fashion that leads progressively from gross, over-all considerations of photoreactivation phenomena to more refined considerations, approaching the molecular level and the particular mechanisms involved. First there is a description of *what* is meant by photoreactivation, what wave lengths of light are involved, and what biological systems are affected. These data are then summarized by a definition of photoreactivation at the end of Section II. This section thus consists largely of a cataloguing of information and treats only incidentally of mechanisms.

Section III describes the macroscopic physicochemical aspects of photoreactivation, with the general object of answering the question of *how* photoreactivation behaves. Section IV concerns the mechanism(s) of photoreactivation, with emphasis on the microscopic physicochemical aspects. First, the machinery involved (Molecular Components) is discussed and then various theoretical models are considered. This section is generally concerned with answering the question of *why* photoreactivation behaves as it does.

In vitro experiments (IV–B) could have been discussed anywhere in the paper. They have been placed in the mechanisms section because of their unique ability to shed light on both the nature of the molecular components and the validity of theoretical models.

II. THE RANGE OF PHOTOREACTIVATION

The description of photoreactivation given in the first paragraph of the preceding section is obviously not precise. To arrive at a clear definition, it is necessary to consider some of the experimental data concerning the range of photoreactivation. In this section (II), these data will be outlined and a definition will be proposed. Hereafter, for simplicity and in order to avoid confusion, photoreactivation will often be referred to as "PR," inactivating light as "UV," and reactivating light as "PR light."

A. Range of Radiations

A rather small range of wave lengths is effective in producing photoreactivable damage. The range of wave lengths of PR light is considerably wider. First, the action spectrum of inactivating light will be considered, that is, the range of UV wave lengths that produce *photoreactivable* damage.

1. Inactivating light. In most experiments on PR, the UV has been of wave length 2537 A. A few investigators have used other wave lengths, and in all cases, in any one experiment, a single wave length of UV was followed by a broad band of PR light. These experiments therefore

[2] Since the "reactivation" is almost always referred to a final measured effect or lesion that is generally irreversible, the term "photoprevention" might be superior. It makes more sense to speak of "photoprevention" of killing or mutation than to speak of "photoreactivation" (or "photorestoration," etc.) of killing or mutation. If this term were used, one would also have to refer to the primary agent and the relative time of treatment. Thus "post-UV-photoprevention" would describe completely what is here called photoreactivation. In addition, this term would distinguish the present effect from photoprotection ("pre-UV-photoprevention") and from possible visible inactivation-reactivation effects ("postvisible-photoprevention"). However, the term "post-UV-photoprevention" is not suitable for the transforming factor and probably not well suited to the crucial photoreactivation events at the molecular level. Because of such complications, decision on this matter is left to the future, when better knowledge of the mechanism will permit selection of a better term.

[3] Short reviews of photoreactivation, included in reviews on broader subjects, have been written by Giese (52), Errera (46), Muller (121), and Stuy (153).

indicate which wave lengths of UV produce damage that can be photoreactivated.

Studies of what wave lengths of UV produce photoreactivable damage and of the relative degree of the reactivation require thorough experiments. A good approach is to obtain complete dose-survival curves for both inactivation and maximum reactivation for each UV wave length. In no case have such complete determinations been made. In the special case in which both the inactivation and the maximum reactivation curves are exponential at all wave lengths of UV, it would be sufficient to report merely the "maximum per cent PR" at a given level of inactivation, or to give the "photoreactivable sector" (for definition of terms, see III-A). This special case, however, has not been observed nor, apparently, even looked for. Most workers have merely reported the "maximum per cent PR" at a certain level of inactivation. In many cases, it is questionable that the amount of light given was sufficient, for *all* UV wave lengths, to produce maximum PR, and in some cases the amount of light may have been too great. Nevertheless, certain experiments were much more thorough than others, the best to date being those of Giese *et al.* (53) and Zelle *et al.* (174, 175). Because none of these experiments is really complete, because different criteria have been used by different workers, and because some of the determinations are open to question in light of the experimental technique, a survey will provide only a general idea of the spectrum of inactivating light.

Wells and Giese (165), using eggs of the sea urchin *Strongylocentrotus purpuratus*, found PR of delay in cleavage after wave lengths from 2450 to 3130 A, but the effect was less pronounced at 2450 A. Kimball and Gaither (99), working with *Paramecium aurelia*, found that retardation of cell division is equally photoreactivable if produced by 2650 or 2804 A, and they suggest that it is the same after 2378 and 2537 A. Carlson and McMaster (28), using embryo neuroblasts of the grasshopper *Chortophaga viridifasciata*, found PR of spheration of the nucleolus after UV of 2650 to 3022 A, but not at lower wave lengths. Fluke (48) reported no change in photoreactivable sector for inactivation of T1 phage between 2400 and 2900 A. Giese *et al.* (53), using the protozoan *Colpidium colpoda*, found PR of retardation of division after wave lengths from 2383 to 3130 A, the effect being less pronounced at 3130 A and below 2537 A. Zelle *et al.* (174) found different photoreactivable sectors for killing and mutation to streptomycin independence in strain SD-4 of *Escherichia coli* but for either property the sector was constant between 2378 and 2967 A. Also, the shapes of the PR curves were similar in this range. At 2180 and 2250 A (175), this strain was sensitized to the PR light, so that the survival rose with dose of PR light to a lower level than with longer wave lengths and then dropped rapidly with increasing dose of PR light (figure 1). Mutation showed a

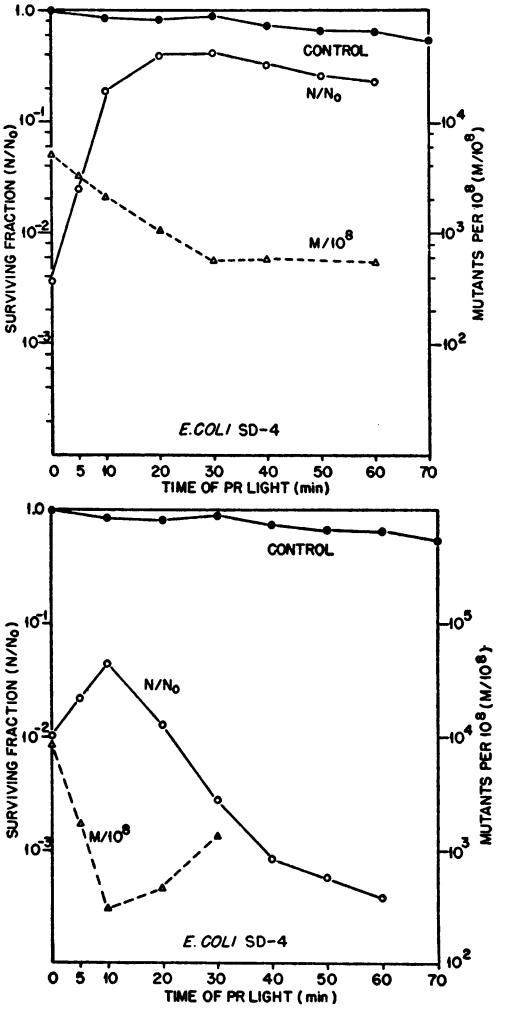

Figure 1. Photoreactivation of killing and mutation (mutants per 10^8 surviving cells) to streptomycin independence after irradiation with (*A*, *top*) 2650 A, and (*B*, *bottom*) 2250 A. (Zelle *et al.* (175).)

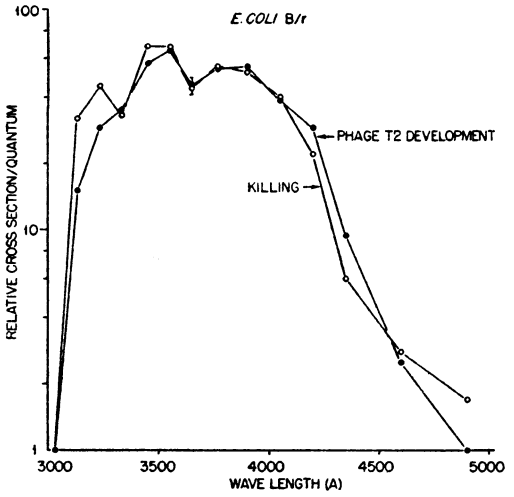

Figure 2. Relative effectiveness per quantum of different wave lengths in producing one per cent photoreactivation. At 3650 A is indicated the standard deviation of the mean for all points in the central part. The curves are identical within experimental error for most of this region. (From Jagger and Latarjet (84).)

similar sensitization at these wave lengths. These experiments, in addition to many others (*e.g.*, figure 3), suggest that the *rate* of PR is not necessarily related to the *maximum* PR obtainable. PR behavior similar to that of SD-4 was found with the related strains B/r and 82/r. Another strain (42-24) of *E. coli*, however, showed the same PR (for killing) after UV of 2652 and 2250 A, there being no evidence of a sensitization in this strain.

Heinmets and Taylor (65) studied the effects on bacteria of light from an electrical discharge. The composition of the radiation is not known, but it probably involves photons below 2000 A, some of which are undoubtedly ionizing. For killing of *E. coli* B and B/r with this radiation, no PR was found.

Several workers have tried PR after X-rays. Dulbecco (38) found that phage T2 inactivated by X-rays in synthetic medium (predominantly indirect effect) showed no PR, whereas phage inactivated in nutrient broth (predominantly direct effect) showed slight PR. He assumes that the observed PR was decreased by the known poor adsorption of the phage after X-irradiation; however, even after this correction, the effect is still small. Similar results were obtained by Watson (158, 159) with T2, T4, and T6. Latarjet (104) reported PR of induction of prophage development in *Bacillus megatherium* after X-rays, but he later found (105) that the observed effect was caused by an arrest of virus development by visible light after X-rays and was not a true reversal of the induction process. Other workers have found that there is no PR after X-rays for induction of prophage development in *Pseudomonas pyocyanea* (154), reduced vigor after autogamy in *Paramecium aurelia* (99), mutation in *P. aurelia*, as measured by death and reduced division rate (98), and cleavage delay in the fertilized egg or inactivation of the sperm of *Arbacia punctulata* (17).

Phage T2 that has been killed by incorporated P^{32} is not photoreactivated inside its host, *E. coli* B/r (149).

Summary. Photoreactivation has been found of damage caused by UV in the wave length range 2180 to 3130 A. The photoreactivation occurs in about the same manner and to about the same extent after UV in the range 2400 to 2900 A. Outside this range, damage is usually less photoreactivable, the lower UV wave lengths sometimes sensitizing to killing with moderate doses of PR light. Experiments immediately outside the range 2180 to 3130 A have not been attempted. (It is difficult to obtain a strong monochromatic beam of UV below 2180 A, whereas above 3130 A the UV itself is usually photoreactivating.) There may be slight photoreactivation after X-rays, but in no case is this a large effect. It appears that damage produced by ionization is not photoreactivable, the slight effect after X-rays probably being caused by reversal of damage caused by excitations only. It is of interest that these remarks hold for a variety of radiation effects in a variety of organisms. Photoreactivable damage, if it is produced at all, seems always to be produced by about the same UV wave lengths, suggesting that the UV chromophore is of the same type in all cases.

The wave lengths effective in producing photoreactivable damage are those that, in most cells, would be absorbed chiefly by nucleic acid and/or protein. In addition, it is generally accepted that the types of damage listed above are caused by nucleic acid or nucleoprotein absorption.[4]

[4] A possible exception appears in the work of Hausser and von Oehmcke (63), who photoreactivated browning of banana skin, an effect show-

The damage is about equally photoreactivable, whether the photon has a wave length such that it is well or poorly absorbed, suggesting that even when these compounds are absorbing in regions other than their absorption peaks, the energy is being channeled in such a way as to produce the same damage. In addition, the constancy with wave length of the photoreactivable sector implies that both the photoreactivable and the nonphotoreactivable damages have similar action spectra and hence that they result from absorption by similar chromophores.

2. Reactivating light. In all the experiments on the action spectrum of the PR light, inactivation was effected with 2537 A. Unlike the experiments mentioned above, many of these action spectra were related to the rate of PR rather than to the maximum per cent PR. This is reasonable, for whereas in a study of the inactivating light one wishes to know the *extent* of the photoreactivable damage produced by a certain UV wave length (maximum per cent PR), here one wishes to know the *effectiveness* of a certain PR wave length (rate of PR). (*A priori* considerations and available data suggest that the rates are more constant in the former case, and that the maximum per cent PR's are more constant in the latter case.) Hence, the action spectrum of PR light is the usual type of action spectrum which, according to Loofbourow (112), should be a plot of "... the reciprocals of the incident energies required at different wave lengths to produce a given biological effect..." Therefore, these spectra are usually determined by measuring the incident dose required to produce a given level of PR. This level should be at a point where the dose-effect curve is linear, and in a dose rate range that shows reciprocity of time and dose rate.

Kelner (92) found that spores of *Streptomyces griseus* showed PR of killing with PR light in the range 3650 to 4940 A, with a single high peak at 4358 A. *E. coli* B/r killing showed PR in the range 3650 to 4760 A, with a single low peak at 3750 A. He did not use wave lengths below 3650 A, even though there was still considerable PR at that point for both organisms. For neither organism was there any measurable PR in the range 5240 to 6950 A. These experiments were done with lines from a medium-pressure mercury arc lamp and bands isolated from an incandescent source. Dulbecco (38), using only lines from a mercury arc, did an action spectrum for PR of killing of phage T2, using *E. coli* B as host, and found high PR only at 3650 and 4047 A, the maximum being at 3650 A. In addition, he observed slight PR at 4358 A and also at 3130 A, where there was some killing by the light, for which a correction was made. Giese *et al.* (53), studying division delay in *Colpidium colpoda*, and using lines of the mercury spectrum isolated with a monochromator, found high PR at 3341, 3650, 4047, and 4358 A, with roughly equal maxima at 3650 and 4358 A. There was no PR with "yellow-green" light. This spectrum was determined using constant doses of light which permitted high but not full PR. Later work by Giese *et al.* (56) showed that *C. colpoda* division delay in starved cells can be photoreactivated over a wider range (3130 to 5490 A). Helmke (67) found *E. coli* B to be reactivated with 3650 and 4358 A, the former wave length being more effective. Stuy (150) found PR of *Bacillus subtilis* and *B. cereus* at 3650, 4047, and 4358 A, the maximum being at 4047 A. Jagger and Latarjet (84), using *E. coli* B/r and the complex T2-B/r, showed (figure 2) that the action spectrum is the same for both the bacterium and the phage, that PR occurs for wave lengths from 3130 to 4600 A, that it is high and relatively constant in the range 3130 to 4200 A, and that there are three small maxima at 3240, 3500, and 3850 A (the phage curve shows only a shoulder at 3240 A). For these spectra, both the lines and the continuous background of a high-pressure mercury arc were used, and narrow bands of high purity were isolated with a double monochromator. This is the most detailed action spectrum that has been done for PR. The only other spectra done with a monochromator were those of Giese *et al.*

These experiments on the spectrum of PR light may be criticized chiefly on the basis of precision and completeness of the investigation. Giese *et al.*, by using constant doses of

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ing an action spectrum similar to that for erythema of human skin (*i.e.*, minimum at 2800 A, maximum at 2970 A). However, Blum *et al.* (14) were unable to photoreactivate sunburn. Furthermore, shielding and other factors do not permit elimination of nucleoprotein as the active absorber for erythema, in spite of the observed action spectrum (see Blum (11) pp. 514–517).

light, must have obtained different levels of PR with different wave lengths, and hence did not satisfy the aforementioned criterion of a "given biological effect." Kelner, and Jagger and Latarjet, measured PR to a constant level, whereas Dulbecco measured the intensity required to produce a standard rate of PR. The latter is an excellent procedure for phages, which show a linear rate starting from zero time, but is of debatable value for bacteria, which show initial lag periods. The work of Stuy and of Helmke was very limited in the number of wave lengths used. Many workers used filters, which are not as good as monochromators for isolation of the light bands. Only Kelner, and Jagger and Latarjet, used bands between the mercury lines, thus obtaining more detailed spectra. In each of these experiments, the spectrum was obtained at only one level of inactivation. It is possible that these levels were poorly chosen in regard to obtaining a spectrum that would identify the principal chromophore, although the observation of Nishiwaki (125) of a constant PR rate in *E. coli* after two different UV doses tends to discount this possibility.

An account of certain remarkable results was published in a brief paper by Cantelmo (27), concerning PR of induction of prophage development in *Staphylococcus aureus*. He found PR with wave lengths 4400, 5500, and 6600 A, the greatest effect being obtained with 6600 A. Only one other investigator has found PR above 5000 A (Giese *et al.*, (56); 5490 A), and no one else has found a maximum above 4358 A, which is 2200 A lower than the maximum of Cantelmo. PR of induction is a tricky thing (see II-A-1 and III-B), as evidenced by the papers of Dulbecco and Weigle (41) and Latarjet (105). In view of this fact, as well as the uniqueness of Cantelmo's results, these experiments are open to serious question. They should be repeated, using the tests outlined by Dulbecco and Weigle.

Summary. Photoreactivation has been observed with PR light in the range 3130 to 5490 A. Below 3130 A, the killing effect of the light undoubtedly masks any reactivation. Most spectra indicate a maximum photoreactivation in the region of 3800 A. Exceptions are the spores of *S. griseus*, studied by Kelner, and the *C. colpoda* of Giese *et al.*, both of which show maxima at 4358 A. The small maxima of Jagger and Latarjet, for *E. coli* B/r and its phage complex, are probably not important.

It is interesting that the spectrum of PR light, unlike that of the UV, is not the same in all organisms. This suggests that there is only one type of inactivation chromophore but more than one type of reactivation chromophore. Analysis in terms of the nature of the reactivation chromophores will be undertaken later (IV-A-1).

B. Range of Organisms and Properties

Photoreactivation has been observed in a very wide range of biological materials and for many biological properties, running all the way from reactivation *in vitro* of genetic properties of nucleic acid molecules to prevention of death in mammals. A broad biological range was evident in the experiments previous to 1953, which are reviewed by Dulbecco (40). Subsequent experiments have, in general, simply filled the gaps. Consequently, consideration will be given here only to those cases (a) where photoreactivation of *new* organisms or properties has been found, and (b) in which photoreactivation has been looked for but not found. Many of these experiments will be discussed in greater detail in later sections.

1. Occurrence of photoreactivation (new experiments). Starting with experiments involving the lowest biological organization, there are several reports of PR of biomolecules *in vitro*, although in only one case is the effect unquestionable. These experiments are discussed in detail in section IV-B.

Bacteriophage. A wider range of bacteriophage PR is becoming evident. Two *Rhizobium* phages have been photoreactivated (102), the phenomenon exhibiting the same characteristics as those found with coliphage by Dulbecco. Phage M5 of *Bacillus megatherium* has a steeper survival curve after wet UV irradiation than after dry UV irradiation. The difference in slope can be made up by PR, but this is the only PR found (50). PR of a temperate phage of *Micrococcus pyogenes* has been reported (29), as well as PR of induction of prophage development in *Staphyloccus aureus* (27).

Bacteria. PR of various properties has been found in a wide variety of bacteria, including killing in *Aerobacter aerogenes* (9), *Chromobacterium violaceum* and *Micrococcus pyogenes* (10), *Erwinia caratovora* and *Proteus* D3 (24), *Azotobacter chroococcum* and, to a small degree, *Azotobacter* "Q" (59). UV damage to constitu-

tive and adaptive respiratory systems in *Azotobacter agile* (61) and killing and mutation in *Serratia marcescens* (87) have also been photoreactivated. Lengthening of the lag period has been photoreactivated in *E. coli* B/r (8). Two reports have dealt with the question of reactivation of specific sites within the cell concerned with ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) synthesis. These are discussed in section IV-A-2. PR in the bacilli is highly variable and will be discussed in II-B-2.

Weigle (162) reported a curious type of restoration of phage by UV-irradiated bacteria, which he calls "UV restoration." The acquisition of this restoring property by the treated bacteria can be reversed by PR (see III-D).

Fungi. Experiments with the fungi have shown PR of killing in *Ustilago maydis* (24) lengthening of the lag period in *Penicillium notatum* (93), killing and mutation in *P. chrysogenum* (137), and killing and retardation of budding in haploid, diploid, triploid, and tetraploid cells of *Saccharomyces* (140). Kelner (93) found PR of dry actinomycete spores that had been UV irradiated dry.

It may be mentioned at this point that many phenomena resembling PR have been reported in the literature. Those prior to 1936 are reviewed by Prat (129). Since then, Whitaker (168) reported PR of retardation of development of rhizoids in the alga *Fucus furcatus*. Kelner, of course, was the first to grasp the significance of PR and to study it in detail.

Protozoa. Killing in *Chlamydomonas moewussi* has been photoreactivated (163). Delay in separation time after conjugation in *Paramecium bursaria* shows PR, whether the UV is given before or after conjugation. The reactivation appears least effective between early and late prophase of the first meiotic division (C. F. Ehret, A. Votava, and L. Harrer, *personal communication*). *P. bursaria* shows sexual activity during light periods and lack of activity during dark periods. This photoperiodism persists in a diurnal cycle in continuous darkness. UV curtails the sexual activity in the dark, but visible light restores the periodic activity (42). UV induction of phase shifts in this system is also photoreactivable (43).

PR of cytoplasmic damage has been recently reported in amoeba and in nerve cells. These are described in IV-A-2. They are the only known cases of PR of cytoplasmic damage.

Insects. PR has been found for lethal and molt-retarding effects of preimaginal nymphs of the milkweed bug *Oncopeltus fasciatus* (166), and for killing, adult fertility, and mutation (as measured by the decrease in number of polar cap cells resulting in functional germ cells) in *Drosophila* eggs (118). Light of 3000 to 4200 A will photoreactivate recessive lethal mutations in *Drosophila* polar cap cells after low doses of UV (1), but will *increase* the mutation after high doses of UV (2). The induction of phenocopies by irradiation of pupae of *Drosophila melanogaster* can be photoreactivated (130). Von Borstel and Wolff (19) demonstrated PR of hatchability of the egg of the wasp *Habrobracon juglandis*. When the nucleus of the egg was physically shielded from UV, no PR was found, which indicates that, in this case, only nuclear inactivation is photoreactivable. Carlson and McMaster (28) showed PR of spheration of the nucleolus in embryo neuroblasts of the grasshopper *C. viridifasciata*. These last two experiments are among the few in which PR of damage to a specific morphological cellular site has been demonstrated.

Higher Animals. Large-scale effects in higher animals have been observed. Rieck (135) found striking PR of forelimb development in larvae of the salamanders *Amblystoma maculatum* and *A. opacum*. In these experiments, only the forelimb was irradiated, and penetration of the UV was considerable. In similar experiments on *A. opacum* and *A. punctatum*, Butler and Blum (25) demonstrated PR of both limb regression and induction of accessory limb structure. Blum *et al.* (12), working with the same organisms, found PR that permitted separation of the UV effects of (a) regression of the irradiated portion of the limb, (b) retardation of regeneration after amputation, and (c) production of abnormalities in the regenerate. Zimskind and Schisgall (177) have demonstrated PR of pigmentation changes in tadpoles of *Rana pipiens* and *R. catesbiana*. Here, the whole animal was UV irradiated. Rieck and Carlson (136) showed that, in albino mice, damage to the ear caused by localized UV irradiation and death of the mouse caused by whole-body irradiation are both photoreactivable. Here is an example of PR of an entire mammalian organism, although it must be remembered that the primary UV damage is only to the surface of the body.

Tumors. Griffin *et al.* (62) made the interesting

observation that induction of ear tumors in Swiss mice is photoreactivable, but only if the PR light and UV exposures are simultaneous. PR light applied *after* ultraviolet increases the tumor induction. However, their UV actually contained considerable PR light. Kelner and Taft (97), using monochromatic UV, found indications (the effect is not statistically significant) that induction of carcinomas in the range 2537 to 3100 A in albino mice is photoreactivable. Although these experiments are of great interest, it is evident that PR of tumor induction has not been clearly demonstrated.

2. Lack of photoreactivation. Lack of PR is in some ways of more interest than its occurrence. There are relatively few known exceptions and some of these would be anticipated (*e.g.*, one would not expect to find PR after highly destructive doses of UV). The remaining exceptions may provide some clues to the mechanism of PR.

Blum *et al.* (13) found that, of five UV effects on eggs of the sea urchin *A. punctulata*, only one, delay of cleavage, was photoreactivable. The nonreactivable effects are cytolysis, fixation (extensive visible internal changes without cytolysis), activation (induced cleavage), and removal of the jelly membrane (a tenuous extracellular membrane). It is difficult to interpret these results, partly because the nonreactivable effects show considerable variability and partly because the dose relationships are not clear. The five effects usually require about the same order of magnitude of UV dose, but some may occur without any UV. Cleavage delay seems to result from nucleic acid absorption, whereas the other effects require shorter wave lengths, and the authors interpret this to mean that only damage to nucleic acid is photoreactivable. It is possible, however, that only cleavage delay is photoreactivable simply because it is the mildest and most specific of the five effects. Kirby-Smith (100) found no PR of chromosome breaks in dry *Tradescantia* pollen caused by 2537 or 2650 A radiation (see also Kirby-Smith and Craig, 101). Again, one suspects that chromosome breakage is simply too drastic an effect to be photoreactivable.

In an abdominal stretch receptor of the crayfish *Astacus trowbridgei* the discharge frequency is increased by UV irradiation, but this effect shows no photoreactivation (54*a*).

Hirshfield and Giese (76), using the protozoan *Blepharisma undulans*, found no PR, as measured by (a) regeneration of the cut cell, (b) division rate, (c) conjugation, and (d) survival of either colored or bleached cells. One would expect the last three effects to be reactivable, considering the work on other protozoa. The authors suggest that the cell pigment, whether bleached or not, has poisonous effects when illuminated with PR light, thus masking PR. Brandt and Giese (22) found, in *Paramecium caudatum*, that division delay is photoreactivable, but immobilization (lack of ciliary movement) is not. Since the former shows a nucleoprotein action spectrum and is presumably a nuclear effect, whereas the latter has a protein action spectrum and is probably cytoplasmic, the authors conclude that only nuclear damage is readily photoreactivated. An alternate conclusion would be that nucleoprotein damage is photoreactivable, whereas other protein damage is not.

The greatest number of known exceptions to PR occur in the bacteria. Johnson *et al.* (86) failed to find PR of killing in *Bacillus cereus*. Stuy (150, 151) found it in *B. mycoides*, *B. pumilis*, *B. megatherium*, and in three strains of *B. cereus*, but did not find it in a fourth strain of *B. cereus*, nor in *B. subtilis*, *B. polymyxa*, and *B. circulans*; however, he quotes Kelner as having found positive results in some cases by altering the experimental conditions.[5] Stuy himself (150) found that the dose rate of PR light was an important factor. Three strains of *B. megatherium* failed to show PR, although some small effects with phage M5 in *B. megatherium* were found (50). PR of induction of prophage development has been observed in *B. megatherium*, but only

[5] Kelner (*personal communication*) states, "I found in unpublished work some years ago that the bacilli show secondary effects of UV which make demonstration of PR difficult. Some of these effects are: continued death when cells are held in the dark in liquid medium after UV, spontaneous recovery under such conditions, sensitivity to PR light (as shown by Stuy), and increased sensitivity to trace toxic materials in the assay agar. When these factors are controlled, PR is generally but not always demonstrable, although there is great variation among strains. The same considerations hold for micrococci. Some bacilli are as highly reactivable as coli. Bacilli, micrococci, and perhaps yeast probably form a group distinct in photoreactivation properties from the coliforms and other gram-negative rods."

after low doses of UV (127). It seems that the bacilli are very sensitive to destructive action by PR light, and this may be why they show such diversity in response.

Bellamy and Germain (9) were unable to photoreactivate *Streptococcus faecalis* and *S. lactis*. Goodgal *et al.* (58) were unable to photoreactivate *Hemophilus influenzae* or *Diplococcus pneumoniae*. Goucher *et al.* (59) could not photoreactivate *Azotobacter vinelandii*, although three other strains of *Azotobacter* were reactivated. *A. vinelandii* showed no killing with either 1 or 30 min exposures to PR light, both of which gave PR with the other species. This indicates that there probably was no killing effect with *A. vinelandii*, although this does not rule out the possibility of a dose rate effect, such as Stuy found.

This large number of exceptions to PR in the bacteria is puzzling. It could be caused by lethal effects of the PR light (150) or the lack of substances required for PR, as suggested by the experiments of Goodgal *et al.* (58). The former explanation seems more tenable in those cases where some species are reactivable and others are not (*Bacillus*, *Azotobacter*), whereas the latter explanation may apply to those families in which no reactivation at all has been found and that are known to have complex nutritional requirements (the Lactobacteriaceae, including *Streptococcus* and *Diplococcus*, and the Parvobacteriaceae, including *Hemophilus*). Tests of other members of these families would be interesting.

Heinmets and Taylor (64) performed a series of experiments with *E. coli* B in the liquid state at 5 C and in the frozen state at −70 C. UV alone killed the bacteria in either the liquid or the frozen state; PR light above 3000 A alone produced killing in the frozen state only. If the UV was applied in either the liquid or the frozen state, subsequent PR light produced further killing in the frozen state, but photoreactivated in the liquid state. Kaplan and Kaplan (88) found no PR of *Serratia marcescens*, if it is given UV in the "dry" state (33 per cent relative humidity). If it is exposed to UV in the "wet" state (97.5 per cent relative humidity), then wet PR is observed for both killing and mutation, but dry PR is observed only for mutation. These experiments are open to some question, since both the normal death rate and the UV sensitivity of *S. marcescens* are very high at 33 per cent relative humidity (88, 119). Stuy (152) found that spores of *B. cereus* are not photoreactivable, even when heated and then placed in a synthetic nongrowth medium that permits uptake of water and transfer of other substances, as well as development of sensitivity to heat and radiation. Only when they were placed in a nutrient medium, and synthesis presumably began, could PR be demonstrated. Romig and Wyss (138) found that sporulating cultures of *B. cereus* lost completely their photoreactivability at the same time that the UV resistance increases. Kelner (93), on the other hand, has reported PR of dry actinomycete spores that had been UV inactivated dry, although the spores were in equilibrium with an almost saturated atmosphere (*personal communication*) and hence undoubtedly contained water. Also, Kelner's original discovery of PR, and some later work (132) was done with spores of *S. griseus* suspended in saline or water and then UV irradiated and photoreactivated. In this treatment also the spores absorb water. Hill and Rossi (72) found that T1 phage, when inactivated with UV in the dry state, is not photoreactivable. However, Fluke (49), using a different drying technique, found that T1 is photoreactivable after dry UV inactivation. In summary, it seems that, in general, UV damage produced in either the liquid or the solid state can be photoreactivated, but the reactivation must occur in the liquid state (see, however, Hill and Rossi (73, 74)).

Phages and sperm show little or no PR outside a host cell. Dulbecco (38) early observed that under no circumstances could infectivity of T2 phage be photoreactivated if the phage were not adsorbed to their host (see IV-B). Wells and Giese (165) found a small PR of delay of cleavage by UV irradiated sea urchin sperm when the sperm were illuminated before fertilization, although the effect seems to be severely limited by lethal effects of the PR light. Blum *et al.* (17), using eggs and sperm of *Arbacia punctulata*, and Iverson and Giese (81), working with the echiuroid worm *Urechis caupo*, showed that the sperm were not photoreactivable outside the egg. Both phages and sperm are easily reactivated after entry into an intact host cell. Dulbecco (38) also showed that heat-killed bacteria will not support PR of phages, whereas ultraviolet-killed bacteria will (40). It seems that phages always and sperm usually require a complex environment if they are to be

photoreactivated. Another possibility is that the "reactivable site" (see IV-A) of a phage or of a sperm is not in the appropriate physical conconfiguration until it enters the host cell.

There are two cases in which transforming factors showed no PR, whether illuminated outside or inside the host bacterium, but that are photoreactivable in extracts of *E. coli*. These are discussed in detail in IV-B. The transforming factor therefore appears to require not only a complex environment, but also one that does not exist in all organisms.

Bawden and Kleczkowski (6, 7) photoreactivated to a small extent the spherical tomato bushy stunt virus in leaves of *Nicotiana glutinosa* and tobacco necrosis virus in French bean (*Phaseolus vulgaris*), but were unable to do so with the rod-shaped tobacco mosaic virus (which has only one-third the RNA content of the other two viruses) in *N. glutinosa* (see IV-A-2). Dulbecco (40) showed a correlation between nucleic acid content and photoreactivability in the T phages. Failure to photoreactivate tobacco mosaic virus could be caused either by its low content of RNA or by an unfavorable physical state of the RNA for production of photoreactivable damage. Both spherical viruses could be reactivated only inside the host cell. It is of interest that systems comprised of DNA and protein (phage), RNA and protein (plant virus), and pure DNA (transforming factor) all can be photoreactivated, although it should be emphasized that the plant virus result is based on a single study in which the effect observed was small.

No clear-cut PR has been found for various relatively simple biomolecules *in vitro* (see IV-B).

Summary. Photoreactivation is a nearly universal phenomenon. It has been found in biomolecules, viruses, microorganisms, and higher plants and animals. All orders of living things that have been tested show photoreactivation; indeed, only in the bacteria have groups as large as families been found that seem not to show this phenomenon. Although in many cases a positive effect might have occurred under different experimental conditions, it does appear that some species actually do not possess the apparatus required for photoreactivation.

Properties that have been photoreactivated include mutation, transformation, enzyme synthesis, morphology, and pigmentation. These properties are in a minority, however, for most effects observed involve the ability to reproduce in a normal manner. Since reproduction, as well as mutation and transformation, is known to be intimately related to nucleic acid or nucleoprotein, the spectrum of effects observed in photoreactivation supports the conclusion drawn from the spectrum of UV wave lengths that produce photoreactivable damage (II-A-1), namely, that photoreactivation is primarily concerned with damage to nucleic acid and/or protein.

Photoreactivation seems to be possible whether the UV damage occurs in the liquid or the solid state. However, the reactivation seems to require not only the liquid state, but a rather complex environment, similar to that within a living cell.

The similarity of photoreactivation in widely differing systems suggests that it is basically the same process in all cases. There is evidence for considerable differences in detail, however, such as in the nature of the chromophore.

C. Definition of Photoreactivation

With all these observations in mind, I shall now attempt to define photoreactivation in a manner consistent with present knowledge and possible new discoveries.

Since PR is effected by wave lengths down to 3130 A, the PR light cannot be called "visible" light. The spectral ranges cannot be specified, since these may someday be extended. The term "nonionizing radiation" cannot be used for the inactivating radiation, since this could apply to reactivation phenomena in the infrared. The term "living system" cannot be used, since the transforming factor and the viruses are not "living" in the usual sense of the word. Nor is it possible to be specific about the type of damage. Although there is almost always damage to the cellular reproductive system, there are well defined cases of PR of nonreproductive cellular properties, such as pigmentation changes in tadpoles.

In the light of these limitations, the following definition seems to be satisfactory at the present time: *Photoreactivation is the restoration of ultraviolet radiation lesions in a biological system with light of wave length longer than that of the damaging radiation.* In this definition, the term "lesions" corresponds to the "observable lesion" defined by Latarjet and Gray (108) as "any particular observable change in a cell (tissue,

organ, or organism), resulting from exposure to radiation," with the slight modification that the term used here applies to biomolecules and viruses, as well as to cells. The term "restoration" is the same as that used by these authors, who define it as a "diminution of the observable lesion resulting from a treatment or procedure which is achieved by treating the cell in some way after the beginning of irradiation."

This definition conforms to the limitations just mentioned. A defect is that the term "ultraviolet" excludes X-ray effects. However, it is probably only the part of the X-ray damage that is similar to ultraviolet damage (*i.e.*, that caused by excitations) that is photoreactivable; the term "damage by excitations" cannot be used since it would extend into the infrared.

The general consistency of the experiments that have been done, in particular the fact that they appear to concern only lesions in which damage to nucleic acid and/or protein is paramount, indicates that the phenomena observed are closely related. This justifies the definition of a single term to describe them. Phenomena discovered in the future that differ radically from the process so far discussed simply will not be photoreactivation in the sense that this term is now used; they will be something else, requiring a different definition.

The choice of the word "photoreactivation," of course, is a different matter. This was discussed in the Introduction.

III. GENERAL BEHAVIOR OF PHOTOREACTIVATION

This section is concerned with the macroscopic physicochemical aspects of photoreactivation, the general object being to answer the question of how photoreactivation behaves. Most of the quantitative work has been done with phage and bacteria, and reference will be made predominantly to these studies. Unless otherwise specified, all reference to inactivation in this section refers to 2537 A radiation.

A. Dose Relationships

The general dose relationships in photoreactivation have been described by Dulbecco (40) but will be summarized here for completeness.

The bacteria may be inactivated and reactivated in liquid medium, or both irradiations may be done with the bacteria on a solid agar surface. The phages are usually inactivated and allowed to attach to the bacteria in liquid medium; then the complexes are photoreactivated in either liquid or solid medium. It is possible for the phage, while inside the bacterium, to be both inactivated and reactivated (40), the doses required for both processes often being lower for the phage than for the host cell. This procedure, of course, involves inactivation effects on the bacterium as well as on the phage, and most workers have preferred to inactivate the phage outside the bacterial cell.

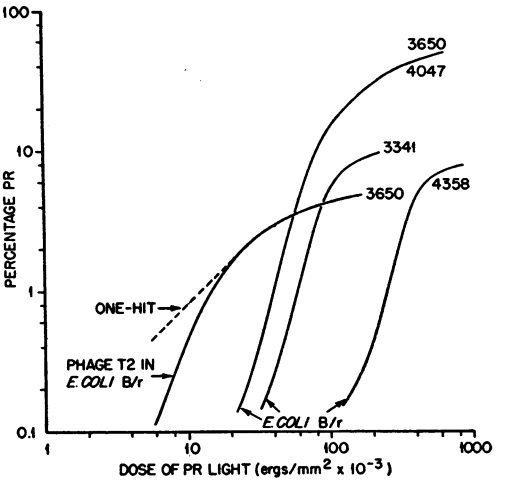

Figure 3. The percentage photoreactivation (% PR) at several wave lengths, plotted logarithmically, as a function of dose of photoreactivating light, plotted logarithmically. The "one-hit" curve agrees with data for phage T2 in *E. coli* B (Dulbecco (38)). (Modified from Jagger and Latarjet (84).)

If N_0 is the total number of viable cells before irradiation, N_D (dark survival) the number of survivors after ultraviolet inactivation, and N_L (light survival) the number of survivors after photoreactivation (figure 4), then,

Fractional light survival
$$= N_L/N_0$$
Percentage light survival (per cent survival)
$$= 100\ (N_L/N_0)$$
Degree of photoreactivation
$$= N_L - N_D/N_0 - N_D$$
Percentage photoreactivation (per cent PR)
$$= 100\ (N_L - N_D/N_0 - N_D)$$

The degree of photoreactivation (92) represents the fraction of inactivated cells that has been photoreactivated. The per cent PR practically

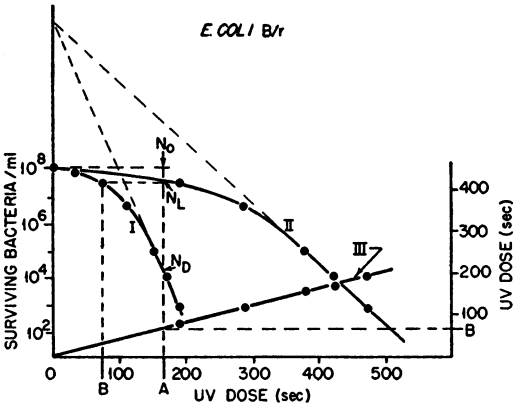

Figure 4. Survival curves: *I*, in darkness; *II*, after maximum photoreactivation. Curve *III* shows the ratio of UV dose at a given dark survival (right ordinate) to the UV dose yielding the *same survival* after photoreactivation (abscissa). A constant dose-reduction factor is revealed by curve *III* being a straight line and curves *I* and *II* extrapolating to the same point on the ordinate. N_0, original number of bacteria; N_L, number of light survivors; N_D, number of dark survivors. (Modified from Novick and Szilard (128).)

never reaches 100, since usually not all the inactivated cells are capable of being photoreactivated.

If bacteria or phage are inactivated to about 10^{-3} survival, and per cent survival plotted on a logarithmic ordinate versus dose of photoreactivating light on the abscissa, it is found that the per cent survival of the bacterium *E. coli* B/r undergoes an initial lag period that is not evidenced by phage T2 in *E. coli* B and is barely discernible with T2 in *E. coli* B/r. The curves then rise in an almost linear way, then curve slowly into a plateau. For this dark survival (10^{-3}), the plateau is lower for the phage than for the bacterium. Plotting per cent PR instead of per cent survival on the logarithmic ordinate minimizes the effect of the lag period, but even so, for different photoreactivating wave lengths, the slope of the central portion of the per cent PR curve differs, and sometimes the level of the plateau will differ also.

If the curves for per cent PR are plotted with both abscissa and ordinate logarithmic (figure 3), the central portion of both the phage and bacterial curves is quite linear (exactly linear with slope of unity for the one-hit phage curve at low doses). More important, the slope usually remains the same for different wave lengths. In action spectrum studies, a good procedure is to compare the doses required to photoreactivate to a level within the linear portion of this curve for all wave lengths.

The curves in figure 4 show dark and light survival for bacteria after *maximum* PR (*i.e.*, each point on the upper curve represents the plateau level of PR at that UV dose). These curves differ for different types of phage and bacteria. They all show some type of initial curvature, after which they all become linear.

Figure 4 shows that, as the UV dose increases, the maximum per cent PR decreases. For low doses of UV, the maximum per cent PR is nearly 100, but with higher doses it drops rapidly. However, as pointed out by Kelner (92), the ratio N_L/N_D increases with UV dose. In any PR experiment, these ratios must be taken into account. At low UV doses, the N_L/N_D will be small, and the number of viable cells before and after PR will be of the same order of magnitude, making it difficult to quantitate results. On the other hand, at high UV doses the maximum per cent PR will be small, and then one will be studying only a small fraction of the population (damage to the rest of the population having become irreversible), and this fraction may be atypical. In work on *E. coli* B/r, a good compromise for most purposes is found at a dark survival of about 10^{-3}. This permits a high N_L/N_D (about 500) and a fairly high maximum per cent PR (about 50).

At the bottom of figure 4 is a curve whose slope is the

$$\text{Dose-reduction factor} = \frac{\text{UV dose for a given survival in the dark}}{\text{UV dose for } \textit{same survival} \text{ after maximum PR}}$$

a concept introduced by Kelner (90). For any one survival, the numerator of this fraction is on the right-hand ordinate in figure 4 (*e.g.*, the dose at *B*), and the denominator is on the abscissa (*e.g.*, the dose at *A*). The curve is a straight line for this bacterium, *i.e.*, one observes a "constant dose-reduction factor" (90, 128). Photoreactivation in this case behaves simply as if it were reducing the ultraviolet dose by a constant factor. For killing in *E. coli* B/r, the dose-reduction factor (DRF) is 0.4, meaning that PR reduces the effect of the UV dose to what it

would be at 0.4 of that dose.[6] With a constant DRF, tangents to the light and dark survival curves at any one survival level will intersect on the ordinate.

A constant DRF exists for PR of killing of *E. coli* B/r (figure 4), and probably of mutation in B/r (90, 128). It is also seen for killing in *E. coli* communis (124) and in *E. coli* B, but in the latter case only for resting bacteria, and not for cells in the logarithmic phase (40). It is not observed for killing in *Azotobacter* (59) nor for the T phages in *E. coli* B (40),[7] whether in single or multiple infection (39). In yeast (*Saccharomyces*) it has been observed for killing, regardless of ploidy (140, 157), but not for retardation of budding (140). In protozoa, it does not occur for killing in starved *Colpidium* (54) and it is questionable that it exists for cessation of division in *Paramecium* (99). Evidently, constant DRF's are rather atypical. This will be further discussed in IV-C-2. However, although the DRF is usually not constant, in most cases it is not far from it; the concept is therefore still valid and very useful in a qualitative way.

In almost all organisms studied, induction of mutations by UV rises to a peak or plateau and then falls off at higher doses. Newcombe and McGregor (122) showed that, in *Streptomyces*, PR at high doses of UV *raises* the mutation level toward the peak, as would be expected in terms of dose reduction. Altenburg and Altenburg (2), however, found that exposure of *Drosophila* polar cap cells to PR light after high doses of UV actually raises mutation *above* the plateau level, suggesting UV sensitization to mutation by PR light.

Another useful term, introduced by Dulbecco (38), is the

Photoreactivable sector = (1 − dose-reduction factor at same survival).

This is the fraction of the total cross section for inactivation that is subject to photoreactivation. Where a constant DRF exists, it is a direct measure of the photoreactivability of a given system. Where a constant DRF does not exist, it is of less value but is still useful in comparing the photoreactivability of different systems at a given survival level. The photoreactivable sector is not to be confused with the maximum per cent PR. For killing in *E. coli* B/r, the photoreactivable sector is 0.6. For low survival of T1 phage in *E. coli* B, it is 0.68; for low survival of T4 phage in *E. coli* B, it is 0.20. The sectors for killing and for mutation in the same bacterium are usually different (87, 174). The sector for phage is somewhat host dependent (40).

It has been mentioned that the per cent PR seldom reaches 100. However, for purposes of analysis, it would be advantageous to have a function that approaches 1 as a limit. Dulbecco (38) has introduced such a function. If $p(L)$ is the number of active particles after irradiation with PR light of dose L, and $p(\infty)$ is the number of active particles after an infinite dose of light (plateau level), then $\log [1 - p(L)/p(\infty)]$ plotted versus dose of light (L), yields a curve expressing the rate at which the *photoreactivable* particles are being photoreactivated. This function is very nearly a straight line with phage T2 in *E. coli* B/r (84), and it is a straight line with T2 in *E. coli* B (38). The linearity of this curve indicates that PR of this phage is a one-hit phenomenon, or a first order reaction. The negative of the slope of this curve is called the *rate of photoreactivation* (38). This rate is proportional to the probability that a given particle will be reactivated by a unit dose. Not all the phage show one-hit reactivation. T3 yields a multiple-hit type curve, with the number of hits proportional to the UV dose (40). The PR rate curve is a straight line for *E. coli* communis, both at 26 C and 37 C, as well as after two different UV doses that give dark survivals of about 2×10^{-3} and 2×10^{-5} (125). The rate curve is not a straight line for *E. coli* B/r (84), showing a multiplicity of hit less than two.

Summary. Photoreactivation can effectively reduce the UV dose by a constant factor. Photoreactivation is seldom a 100 per cent effect. In some phage and bacteria it is very nearly a

[6] Care should be exercised in using the term "dose-reduction factor." Unless the level of dark survival is stated, a constant dose-reduction factor may be implied, which means that complete survival curves for both inactivation and reactivation have been obtained and found to be related by a constant factor. Also, some workers have reported DRF's greater than 1. By definition, a "reduction factor" must be less than 1.

[7] Perhaps it would hold for the phages in strain B/r. The work of Dulbecco, next paragraph, and others (Jagger and Latarjet (84); Friedman (50); Goodgal *et al.* (58)) indicates that phage PR is governed to some extent by the host.

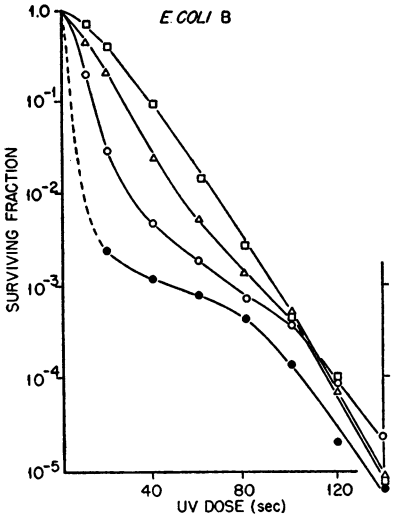

Figure 5. Ultraviolet survival of *E. coli* B after photoprotection by exposure to 0 (●), 1.5 (○), 2.5 (△), and 4.0 (□) min of visible light. (Weatherwax (160).)

one-hit phenomenon but most systems show more complex behavior.

B. Time Relationships

Numerous workers have attempted to produce reactivation by preillumination, that is, to show a protection, rather than a restoration, with light of longer wave length than the inactivating light. In line with the terminology of Latarjet and Gray (108), such effects will be referred to as *photoprotection*. Kelner (89), in his first report of photoreactivation, described attempts to photoprotect killing of *Streptomyces griseus*. No effect was found. In a later paper (90), he reported that there is no photoprotection from killing in *E. coli* B/r. Other workers have reported lack of photoprotection from delay in cleavage in sea urchin eggs (15), from induction of phenocopies in *Drosophila* (130), of survival time after ultraviolet in salamander larvae (16), or from retardation of division in a protozoan, using a moderate dose of 4358 A light (53). Bawden and Kleczkowski (7) showed that illumination of plants before infection with inactivated spherical viruses did not reactivate the viruses. Dulbecco (38) showed that illumination of *E. coli* B, for long periods up to the moment of addition of UV-inactivated T2, produced no effect. Since addition of the inactivated phage is essentially the same as giving a UV exposure to the complex, this shows that, in this system, there is no photoprotection even if the light is given a few seconds before the UV treatment. Thus, a considerable number of experiments have failed to show any significant photoprotection.

Weatherwax (160), however, reported a large photoprotection in *E. coli* B (figure 5), using a high-pressure mercury arc source with the far UV filtered out. The phenomenon involves a gradual change, with increasing dose of photoprotecting light, from the strain B logarithmic survival curve to the strain B/r type of sigmoidal survival curve. Preillumination raised the survival as much as from 10^{-3} to 10^{-1}, a truly remarkable effect. Only a few minutes were allowed to pass between the photoprotection treatment and the inactivation, and the effect was not obtained with B/r under identical conditions (*personal communication*). Although many treatments are known that will change the shape of the survival curve of B to approximately that of B/r (see III-D), the fact that the effect found by Weatherwax involved identical conditions in all cases, except for the preillumination, leaves little doubt that this is a true photoprotection. Whether it involves the same mechanism as photoreactivation is another question. Here arises the possibility that preillumination affects some other mechanism in the cell, leading to the ubiquitous change from the B to the B/r curve shape. Had the effect been found with B/r, which also shows high photoreactivation, such questions could be discarded.

Other less well defined cases of photoprotection have been reported. Although Giese *et al.* (53) did not find an effect on retardation of division in *C. colpoda* using blue light, Giese *et al.* (54) did find about 35 per cent photoprotection by exposure to daylight for 4 hr, with no difference observed between starved and unstarved cells. This is a large effect, although not of the magnitude of that found by Weatherwax. No survival curves for the effect are published.

Cantelmo (27), working with induction of prophage development in *Staphylococcus aureus*, reported a photoprotection. The bacteria, illuminated with a 1000-W tungsten lamp at 37 C in nutrient broth, showed high photoprotection *two hours* later, during which time there had been some growth in the nutrient medium. Fresh bacteria, placed in broth that had been illumi-

nated in the presence of bacteria, showed no photoprotection, which rules out the possibilities of light producing a protecting substance in the broth or of its inducing the excretion of protecting substances by the bacteria.

Dulbecco and Weigle (41) offer a plausible explanation of Cantelmo's results. They found that the phage-growing capacity of *E. coli* is inactivated by visible light much more quickly than is the colony-forming ability. This holds for lysogenic as well as other strains (K12 and B). Consequently, preillumination of a lysogenic bacterium would be expected to reduce its ability to support phage growth and hence to lower the amount of induction observed after UV. The net effect would appear to be a photoprotection from induction by UV. The photoprotection from induction reported by Cantelmo cannot be accepted until the tests described by Dulbecco and Weigle have been carried out.[8]

Photoprotection apparently exists, although the only clearly documented case is that of Weatherwax. His results are of great interest and they should be followed up. It is curious that Giese *et al.* found photoprotection with sunlight but not with blue light. This suggests that either near UV or red light may be involved, and the nature of the sources used by Weatherwax and Cantelmo does not eliminate these possibilities. In this event, the mechanism would not be the same as for photoreactivation, in which blue light is always effective.

Photoreactivation would be expected to occur when the visible light is administered at the *same time* as the ultraviolet, unless long lag periods were involved (in many cases, no lag is observed—see this section and IV-B). PR produced by simultaneous exposure to both UV and visible is reported in the early paper of Whitaker (168), dealing with PR of retardation of development of rhizoids in the alga *Fucus furcatus*, which shows a *higher* reactivation for the simultaneous treatment, and by Griffin *et al.* (6.) who found that *only* simultaneous exposures produce PR of ear tumors in mice.

Helmke (66) got PR of killing in *E. coli* B with simultaneous exposures to UV and PR light. The reactivation rate is about two-thirds of that with consecutive irradiations, as would be expected if there were no lag in photoreactivability.

The *rate of loss of photoreactivability* has been studied by many workers. After inactivation, PR in *nutrient* medium of the complex of phage T2 and *E. coli* B at 28 C is possible for about 20 to 30 min (38),[9] of *E. coli* B/r at 37 C for about 2 to 3 hr (90), and of *Saccharomyces cereviseae* at 28 C for about 30 hr (55). In *non-nutrient* medium, photoreactivability usually lasts for a longer time. Thus, the maximum per cent PR of the complex of T2 in *E. coli* B, starved in saline at 37 C, is constant for 70 min after irradiation (38). Both *E. coli* B/r, starved in mineral medium at 37 C and T2 in this host show only slight changes in photoreactivability at the end of 1 hr (84), although for the host a steep drop begins after about 2 hr (Jagger and Latarjet, *unpublished data*), resulting in almost complete loss of photoreactivability at the end of 4 hr (33). *E. coli* communis in saline retains full photoreactivability at 37 C for 3 hr (125). Lowering the *temperature* greatly increases the duration of photoreactivability. *E. coli* communis in saline shows no loss of photoreactivability at 11 C for 5 hr (125). T2 in *E. coli* B/r, in a mineral medium at 5 C, has the same PR rate after 8 hr (84), and there is no loss of photoreactivability of *E. coli* B/r at 4 C even after 24 hr (33). *S. cereviseae*, plated on nutrient agar and held at 5 C, shows only slight loss of photo-

[8] Dulbecco and Weigle showed that white light *after* induction lowered the amount of induction much more than white light *before* induction, and therefore concluded that photo*reactivation* does exist for induction. However, Latarjet found (see II-A-1) that the X-ray-induced bacterium-phage complex was far more sensitive to white light than before X-rays. This could be the case also with UV. Such a sensitization by UV to visible light usually cannot be detected, however, since it is masked by PR. That it sometimes exists is shown by the experiments of Stuy (150) and Nishiwaki (124). If such an effect operated, the "photoreactivation" observed by Dulbecco and Weigle could also be an artifact, caused by increased sensitivity to white light of the bacterial phage-growing capacity after UV as compared with before UV. Latarjet (106) has, however, shown that PR of induction after UV probably does occur, since he observed not only less induction after visible light but also a large increase in the number of colony formers. Since the inducing dose of UV produces only slight killing, these colony formers did not represent PR of killing.

[9] The latent period for phage reproduction at 37 C in this system is about 30 min.

reactivability after 30 hr (55). *E. coli* B, inactivated at −55 C, then held for as long as 6 days at −10 C, and then melted for visible irradiation, retains full "ability to recover" (64). Spherical plant viruses have been kept for 14 days after inactivation with no loss in photoreactivability (7).

Thus the time after inactivation in which PR may still be effected seems to depend on metabolism. If there is no metabolism, normal PR appears to be possible for an indefinite time. If there is metabolism, the period in which PR may be effected is limited. For metabolizing bacteria, with increasing time after inactivation, there is exponential decline in maximum per cent PR, reaching zero in 2 to 3 hr (90). For phages in metabolizing bacteria, the maximum per cent PR also decreases roughly exponentially with time after inactivation, reaching zero in 20 to 30 min, but the *rate* of PR is constant throughout this time (38). Since, at the dose rate used, at least 20 min exposure is required for maximum per cent PR, the decrease in maximum per cent PR is caused by a limitation in the time during which PR can be effected, this time being 20 to 30 min. In other words, photoreactivability in this system remains constant for a certain time and then drops quickly to zero.

Matney *et al.* (117) have succeeded in obtaining a workable degree of mutation to streptomycin resistance in *E. coli* B/r with doses so low (4 erg mm^{-2}) that there is no measurable killing. The mutations are almost completely photoreactivable, but retain this reactivability only 15 to 20 min after UV at 37 C, thus behaving much like a phage. The absence of killing in this system should facilitate studies of photoreactivation of mutation.

Bowen (20, 21), working with phage T2 in *E. coli* B, showed with the use of short (5 sec) light flashes that, although no reactivation can be obtained by illumination of a phage or cells or both before attachment of the phage, the ability to be reactivated after attachment develops without observable lag (less than 5 sec). However, the maximum reactivation rate is obtained only after several minutes. Subsequent experiments (*personal communication*) showed that injection of the T2 DNA into the host cells is necessary before PR can occur, since agents that block injection (cyanide, absence of oxygen, low temperature) also prevent development of the ability to be photoreactivated, although they do not prevent PR if injection has already taken place. The kinetics of injection indicate that injection is completed considerably before maximum reactivability has developed, suggesting that some additional step must take place after injection before PR can occur.

Cell division is probably one cause of loss of photoreactivability. The 3-hr limit observed in *E. coli* by Kelner (90) corresponds with the division time for dark survivors. *Colpidium* can be photoreactivated only up to the time of the first division of the dark survivors (53). Photoreactivability of *Arbacia* eggs is maximal at about 10 min after UV, corresponding to the time of onset of prophase (116), and then falls off as the normal rhythm of cell division is approached (15). On the other hand, Brandt (21*a*) has found considerable PR after division in both *Didinium* and *Paramecium*. In addition, such things as loss of photoreactivability by starved bacteria in about 4 hr at 37 C indicate that metabolism is also involved.

Giese *et al.* (55) showed that starvation of yeast (*S. cereviseae*) for 6 days causes a drop in photoreactivability to about 10 per cent of normal if carbohydrate is withheld, and to about 1 per cent if nitrogen is withheld. Addition of glucose in the first case increases photoreactivability in a few hours by a factor of 5, but the effect of nitrogen starvation seems to be irreversible.

An *increase* of photoreactivability with time has been reported by Giese *et al.* (53) for division delay in *C. colpoda*. The effect increases up to 4 hr after irradiation, at which time the increase is about 30 per cent. Such an effect has never been observed in other systems, and the lack of reported controls permits a possible spontaneous restoration, perhaps similar to heat restoration.

Summary. Photoprotection apparently exists, although there is only one clear-cut case of this, and it may be a quite different phenomenon from photoreactivation. Little is known of the time before inactivation in which photoprotection is possible. The time after inactivation in which photoreactivation may still be effected seems to be limited by cell division and to depend on metabolism. With no metabolism, the time is indefinite, whereas with normal metabolism it is about 2 to 3 hr for bacteria and about ½ hr for phage inside bacteria. Injection of phage DNA into the host is necessary for photoreactivation of the complex, but this is completed some

minutes before the full photoreactivation rate is attained. Starvation in yeast causes a loss in photoreactivability, with nitrogen starvation being most serious.

C. Modifying Factors

A great many factors have been found to influence ultraviolet inactivation, especially in the bacteria (Zelle and Hollaender (173); Jagger and Stapleton (85)). In addition many factors influence photoreactivation. Consequently, one must be most careful in the design of quantitative experiments. The discussion here will concern chiefly those factors that modify photoreactivation, but one should also control factors that are known to be of importance in all biological experiments with light (Hollaender (78, 79)).

The irradiation aspect will be considered first. Dose of UV is commonly measured in incident erg mm^{-2}. This unit is also useful in the visible range. It is strongly recommended that at least one reference dose be given in terms of this or a similar unit, instead of a unit, such as "seconds of exposure," that may be difficult or impossible to translate into energetic terms. Even if only an order of magnitude can be given, this is much better than no energy estimation at all (for measurement techniques, see Hollaender (78, 79)).

The *dose rate* of the *inactivating* light is usually not important, since most UV effects show reciprocity of time and dose rate. The dose rate of the *reactivating* light is important, however. Dulbecco (38) showed a strong dose rate effect at 37 C in phage T2r (figure 6). Comparison with the results of Jagger and Latarjet (84) indicates that the unit used on this figure corresponds to roughly 40 erg mm^{-2} sec^{-1} of incident light at 4047 A. Thus, for this phage, reciprocity of time and dose rate holds only for incident dose rates less than about 10 erg mm^{-2} sec^{-1}. Kelner (92) reported no difference in PR at 37 C over a 71-fold range of dose rate at 4358 A with *S. griseus* spores. For *E. coli* B/r at 37 C, Kelner (92) reported a 30 per cent increase in energy required for a 10-fold decrease of dose rate at 3650 A, whereas Novick and Szilard (128) found only a slight failure of reciprocity with light from a tungsten lamp, and Jagger and Latarjet (84) found only slight differences at 4047 A if the dose rate is in the range 10 to 180 erg mm^{-2} sec^{-1}. Failure of reciprocity in this case may be caused by loss of photoreactivability with time after inactivation. Kelner photoreactivated unwashed cells in saline,

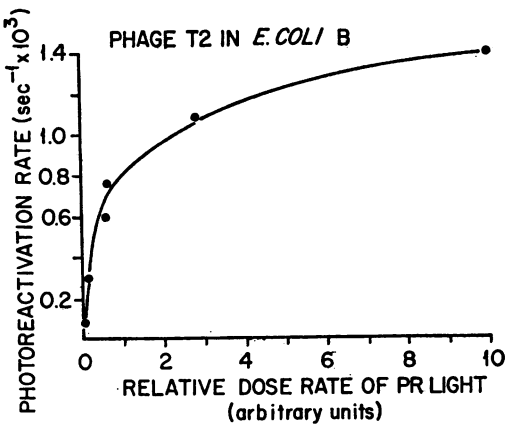

Figure 6. Photoreactivation rate versus relative dose rate of photoreactivating light at 37 C. (From Dulbecco (38).)

a procedure involving considerable loss in photoreactivability with long exposures (90). Novick and Szilard, and Jagger and Latarjet used starved cells, reactivation in the latter case having been carried out in the same synthetic medium (less sugar) in which the organisms had been grown, a procedure involving only slight changes in photoreactivability during long exposures. The bacteria appear to show better reciprocity than the phages.

Some dose rate effects may be caused by lethal action of the light. Stuy (150) found that PR of certain bacilli had not previously been found because the dose rate had been so high that lethal processes overwhelmed the processes of reactivation. He found clear PR in some cases when the dose rate was reduced to about 20 erg mm^{-2} sec^{-1}. The killing of *B. cereus* as a function of dose of white light is shown by Romig and Wyss (138). It has long been known that bacteria are killed, though inefficiently, with light all the way up to 7000 A (113). Studies have been made of killing in *E. coli* with 3500 to 4900 A (77), and in phage with 3400 to 6000 A (156). Both killing of the bacterium and its capacity to permit phage development have been studied in *E. coli* B and K12 with visible light (41), and in *E. coli* B and B/r with 3200 to 4600 A (71). PR of the complexes *E. coli* B-T2 and K12-T2, and the bacterium K12 (when apt to induction), has been shown to occur in inorganic medium with 3400 to 5500 A, but is masked by killing in organic medium (109). Although the killing effect of near UV decreases rapidly as the wave

TABLE 1

Q_{10} for photoreactivation

System	Q_{10} (3–15 C)	Q_{10} (15–37 C)	Reference
Escherichia coli B		~2.5	Johnson *et al.* (86)*
T2 phage in *E. coli* B	7	2.1	Dulbecco (38)*
Transforming factor in extract of *E. coli* B	3	1.4	Rupert *et al.* (139)*
Escherichia coli B/r		1.5	J. Jagger and R. Latarjet (*unpublished*)
		~2	Kelner (91)
Escherichia coli communis		1.3	Nishiwaki (125)*
Streptomyces griseus		~2	Kelner (91)

* The Q_{10} has been calculated from the data of the author(s).

length increases, it has been shown that, in the bacterium *Serratia*, the relative mutagenic effect is considerably greater with 3100 to 4000 A than with the far UV (87). In PR experiments (except those on action spectra) it is wise to eliminate all wave lengths below 3650 A if one wishes to obtain unequivocal results. A further consideration is pointed up by the experiments of Nishiwaki (124), who showed that the killing effect of visible light on *E. coli* communis is increased by previous UV treatment. The form of the dependence on UV dose is sigmoidal, leveling off at survivals lower than about 10^{-4}.

All PR phenomena that have been examined show a *temperature* coefficient greater than unity, thus indicating the general existence of chemical dark reactions. The Q_{10} values are shown in table 1. In the two cases where low temperatures have been studied, there appears to be a break in the region of 15 C, indicating that different reactions predominate above and below this temperature. The agreement among the high temperature Q_{10}'s suggests that the reactions are similar in the various systems. These reactions would have a total activation energy of 5000 to 16,000 cal/mole. Bowen (20, 21) (see also Dulbecco (40)) found that the dark reactions with phage T2 must precede the light reaction, their function apparently being to supply some factor that is used up in the light reaction, and he suggested the following scheme:

$$M \rightleftharpoons N \xrightarrow{h\nu} N^* \rightarrow PR$$

M and N are molecules in equilibrium, but only N can be activated by light to produce PR. Bowen found the time constant for supply of N in the dark to be 35 sec at 37 C, and 9 min at 0 C. The activation energy for the total dark reaction was found to be 9000 cal/mole above 20 C and 17,000 cal/mole below 20 C. Goodgal (57) found that, for survival of microconidia of *Neurospora crassa*, the energy of activation is constant with temperature, but depends on the nature of the radiation source, being 8500 cal/mole for a fluorescent lamp, 14,000 cal/mole for a 1000-W tungsten lamp, and 18,000 cal/mole for sunlight. Goodgal's figure for the tungsten lamp is close to the average of the two values found by Bowen, who used the same light source. Why the value differs for different sources is not clear. Conceivably, light from sources of different spectral composition could be absorbed by different chromophores, and thus set into play different dark reactions. It is difficult to resolve these differences in activation energy, partly because this energy is finely dependent on Q_{10} and partly because few precise data are available. It seems likely that all *E. coli* systems have roughly the values found by Bowen for T2 in *E. coli* B, namely, about 9000 cal/mole at high temperatures and about twice this at low temperatures.

Christensen and Giese (30), studying changes in division pattern of the protozoan *Tetrahymena pyriformis*, found with flashing light experiments that the light reaction time was less than 0.0025 sec and the dark reaction time at room temperature of the order of 0.02 sec. This dark reaction time is similar to that found in photosynthesis (70), but is only 10^{-3} of that found by Bowen for PR of phage. In addition, they found no reciprocity of time and dose rate with 4358 A in the range 35 to 450 erg mm^{-2} sec^{-1}. These data suggest that the mechanism of PR in protozoa may be basically different from that in phage.

Heinmets and Taylor (64) found no PR of *E. coli* B that had been frozen in saline at −70 C. Presumably, this effect was caused primarily by the bacterium's being in the solid state, and not simply by the low temperature.

The *physical state* of the thing being photoreactivated is important. Generally speaking, dry or frozen things cannot be photoreactivated in this state (see II-B-2). Since dark reactions are known to be important in photoreactivation, it is not surprising that the liquid state is required for efficient reactivation.

No *oxygen effect* in PR has been observed with phage T2 in *E. coli* B (38), with *E. coli* B (86), or with *S. cereviseae* (55). In the last two cases, the very sensitive extinction of bacterial luminescence was used to demonstrate removal of oxygen, and in the last case 100 per cent oxygen was also used. A. J. Sbarra and A. Hollaender (*personal communication*), using 10^{-3} M sodium hydrosulfite as a "getter" to remove all traces of oxygen, found, in a suspension of *E. coli* B/r with light of 3500 to 4900 A, a slightly lower PR with air and a considerably lower PR with pure oxygen. Since 3500 to 4900 A light has a killing effect that is enhanced by oxygen (R. L. Gilfillan and A. Hollaender, *personal communication*), this might account for the decrease in observed PR with increasing oxygen concentration. At any rate, the effect, even comparing no oxygen and pure oxygen, is small. The important thing is that oxygen does not *increase* PR, and thus whatever PR reactions are involved, oxygen is not necessary for their functioning.

Respiration machinery may be involved to some extent in photoreactivation, even though there is no oxygen effect. Dulbecco (38) found that cyanide at 10^{-2} M does not affect PR of phage T2, and Giese *et al.* (55) found no effect either of 10^{-2} M cyanide or of azide in PR of yeast. Berger *et al.* (10), however, found that sodium azide, a mutagen and respiration inhibitor, applied before UV irradiation of bacteria, resulted in less killing and less PR; applied after irradiation with UV, there was the same killing but less PR. In terms of dose reduction, the effects observed were small. Nevertheless, these experiments suggest that respiratory systems may have some role in photoreactivation.

A small *pH effect* was observed by Johnson *et al.* (86), working with *E. coli* B at 37 C, who found that at pH 5.4 the maximum per cent PR was slightly lower than at pH 6.9, the curve reflecting a slight inactivating effect at the lower pH.

Physiological aging of cells of *E. coli* B has been shown to decrease their photoreactivability (68). However, *Tetrahymena*, in which cell division has been synchronized by heat treatment, show a maximum PR of division delay that is independent of physiological state of the culture, although the UV sensitivity varies considerably (82*a*).

Starvation effects are discussed in III-B.

Summary. Conditions of irradiation are of the utmost importance. The dose rate of the inactivating light is usually not important, but that of the reactivating light is, especially for phage. Killing and mutagenic effects of the reactivating light may be important. Photoreactivation shows a temperature coefficient greater than one in all cases, indicating the existence of chemical dark reactions. There is no oxygen effect, but respiration machinery may nonetheless be involved. Other factors known to affect photoreactivation are physical and physiological state of the cells, and pH of the medium.

D. Comparison with Other Restorations

A great many protective and restoring effects with ultraviolet have been discovered within the past decade, especially in the bacteria. These effects warrant some attention, partly because they can interfere seriously with photoreactivation experiments and partly because some have shown a relation to photoreactivation. Only the bacterial factors that are known to bear in some way on photoreactivation will be considered here.

In the bacteria, conditions *before irradiation*, such as composition of the growth medium, the stage of growth at which the cells are irradiated, the moisture content of the cells, and the presence of certain chemicals in the medium, may affect survival. Conditions *after irradiation*, such as temperature, composition and pH of the plating medium, time of holding before plating, and presence of chemicals in the holding medium, may also affect survival. Fortunately, UV inactivation of bacteria is relatively insensitive to conditions *during irradiation*, showing no oxygen effect in the liquid state, and no dose rate, temperature, or freezing effects, but some effects of drying. Most of these factors are critically discussed in the review by Zelle and Hollaender (173); more recently discovered ones are included

in a comparison of the effects by Jagger and Stapleton (85).

A fundamental difference between photoreactivation and some other restorations is the degree of restoration with varying degrees of inactivation. In PR, either constant dose reduction or something not too far from it usually occurs, entailing a steady decrease in maximum per cent PR as the UV dose increases. Anderson (4) showed that, with increasing UV dose, heat restorability is lost more rapidly than photoreactivability. This suggests that heat restoration operates chiefly on a more delicate, or earlier, damage than does PR. Catalase restoration (105) on the other hand, is effective only at low survival levels, suggesting that it operates on a later damage (presumably caused by organic peroxides) than does PR.[10] Catalase restoration is presumed to be linked to the peroxidasic rather than the catalatic activity of catalase (106).

Weigle (162) reported that *E. coli* K12S or C, inactivated with UV, X-rays, or nitrogen mustard, supports growth of UV-inactivated phage *lambda* better than do normal bacteria. This "UV restoration" is an effect of the same order of magnitude as PR, and the acquisition of this restoring property by the bacterium can be reversed by PR. Weigle divided the UV damage to the phage into four types: (a) not reparable by either UV restoration or PR, (b) reparable by both, (c) reparable by PR only, and (d) reparable by UV restoration only. Only damages of type d are associated with mutations.

Strains B and B/r of *E. coli* are both highly photoreactivable. However, many of the other restorations act only slightly, or not at all, on strain B/r, often changing the form of the survival curve from that of B to that of B/r (85). This suggests that PR is working on a considerably different damage from that operated upon by the other restorations.

Addition of acetate to the plating medium produces a marked restoration in *E. coli* B and *Corynebacterium bovis* (45). Preliminary experiments indicate that PR and acetate restoration are additive.

Thus there are four lines of evidence that the photoreactivable damage differs from that operated upon by certain other restorations. However, there is further evidence that some of these damages may be similar to the photoreactivable damage.

Duration of photoreactivability in metabolizing bacterial systems at 37 C is about the same as for heat, catalase, and pH restoration (161); namely, 2 to 4 hr. This suggests that all these restorations act on the same kind of damage in the cell, since the reversibility of this damage has about the same decay time in all cases. It is likely that cell division is a limiting factor, but metabolism is also involved (see III-B).

Two reports show a further similarity of heat restoration and PR. In *E. coli* B (148) it is found that PR raises the survival level to that of heat restoration (at 44 C), after which both survivals remain parallel. In *S. cereviseae* (55), heat restoration raises the survival level to that of PR, after which both survivals are parallel.

Stein and Laskowski (148) performed some interesting experiments on heat restoration and PR after inactivation of *E. coli* B with hydrogen peroxide-treated broth. Such broth presumably contains organic peroxides, which produce the inactivation. Their results indicate that UV inactivation does not operate to a great extent through peroxide inactivation, in agreement with the observations on catalase restoration. The over-all picture in this case suggests that PR accomplishes the same thing as heat restoration of either peroxide-broth or UV inactivation and, consequently, that it operates on the same type of damage.

A neighbor restoration has been reported by Delaporte (35). She finds that UV-inactivated cells of *E. coli* B are much more likely to *grow* if they are in clumps with other cells than if they are isolated, and suggests that the cells in clumps have restored one another (neighbor restoration). PR raises the growth frequency of isolated cells by a factor of 9 but does not affect the cells in groups (36). She therefore postulates that PR accomplishes the same thing for isolated cells as neighbor restoration does for clumped cells. Experiments by Galperin and Errera (51) showed that clumping does not increase the probability that any one cell will produce a macrocolony. Therefore, *survival*, as measured by colony count, is not affected by neighbor restoration, but individual colonies are presumably caused to have a multicellular origin.

[10] Catalase restoration is sometimes, but not always, greatly increased by the addition of light in doses too small to effect any significant photoreactivation. This effect is not understood.

Summary. There is some evidence for photoreactivable damage differing from that involved in other restorations and some evidence for similarities. The closest relationship appears to be that between PR and heat restoration.

IV. THE MECHANISM OF PHOTOREACTIVATION

This section concerns the mechanism(s) of photoreactivation, with emphasis on microscopic physicochemical aspects. First the machinery involved is discussed and then various theoretical models are considered. Discussion of *in vitro* experiments is sandwiched between these two subjects because of its obvious bearing on both of them.

A. Molecular Components

Although photoreactivation has, in one instance, been clearly demonstrated in a nonliving cell extract, the phenomenon is of interest chiefly because it concerns functions characteristic of living cells. Consequently, an analysis of its microscopic aspects must be concerned with cell architecture.

The *chromophore* is the site of absorption of the photoreactivating light. Strictly speaking, the chromophore may not be an entire molecule, but only a part thereof, such as a benzene ring or a conjugated carbon chain. However, since so little is now known of the chromophore(s) for photoreactivation, it is not useful to distinguish between chromophore and chromophore molecule. These terms will generally be used interchangeably in this review.

The energy will be absorbed and distributed within the chromophore within 10^{-13} sec. This energy may then (a) be used immediately at the chromophore site, (b) be transferred to another part of the chromophore molecule, or (c) be transferred to adjacent large molecules. This energy transfer and the subsequent primary chemical reactions will occur in 10^{-9} sec (108). Further chemical or physical processes may then eventually accomplish the transfer of the energy, now in a chemical form, to a site where the important biological effect occurs. This site may be called the *reactivable site*; it is a crucial structure within the cell that suffers ultraviolet damage, directly or indirectly, that damage being photoreactivable.[11] If the reactivable site is not subjected to reactivation, it will give rise to the *observable lesion*, which may be at the reactivable site or somewhere else in the cell.

1. The chromophore. The *location* of the chromophore is probably, under normal conditions, always within the living cell (IV-B). Three experiments exist that cast light on its presence in cytoplasm or nucleus. Blum *et al.* (17) reported PR of delay in cleavage when the inactivated sperm of *Arbacia punctulata* is introduced into the enucleate half (obtained by centrifugation) of an egg, and the combination is illuminated with visible light. The sperm alone is not photoreactivable. This shows that a chromophore for PR probably exists in the cytoplasm of the egg. (An alternative explanation would be that the chromophore is in the sperm, but that the sperm is not in a photoreactivable state until its entry into the egg.) PR is also found after the sperm enters the nucleate half of the egg. This, however, indicates nothing about the location of the chromophore, since the nucleate half contains considerable cytoplasm.

A more clear-cut result was obtained by Skreb and Errera (145), who studied survival of nucleate and enucleate halves (obtained by cutting with glass thread) of *Amoeba proteus*. PR was observed in both halves, and the effect appears to be of about the same degree in both cases. In this system, there is no doubt that a chromophore exists in the cytoplasm.[12] The experiment, however, does not indicate whether one exists in the nucleus, since the nucleate half contains about

[11] This definition, of course, depends on what one considers the "crucial structure." At the present time, only an intuitive meaning can be attached to this; the crucial structure is probably always a large molecule or a small group of such molecules. It must be noted also that the "reactivable site" may not be reactivable at all. Thus, if PR involves neutralizing a poison, the reactivable site is the structure that would be damaged if there were no PR; in short, it is the site that appears to be photoreactivated. It may seem that one is here entering a semantic jungle. This is partially true, but inevitable, and arises because of the possibility that PR might not involve any reactivations at all on the molecular level.

[12] In a consideration of the chromophore, the property that is photoreactivated must be kept in mind. PR of "survival" of a cytoplasmic fragment may involve a quite different, and perhaps simpler, mechanism than PR of survival of a whole cell which implies complete return to a normal, dividing state.

the same amount of cytoplasm as the enucleate half. Pierce and Giese (131) have found PR of decreased action potential and sensitivity of frog and crab neurons. These fibers contained no nuclei and little or no DNA. Although the effect is small, this is another case in which the chromophore must reside in the cytoplasm. Thus there is clear proof of the existence of PR chromophores in the cytoplasm but as yet there is no proof of their existence in the nucleus.

These are the only experiments that cast light on the location of the chromophore. Most experiments dealing with the "site of photoreactivation" are concerned with the reactivable site.

In a discussion of the *nature* of the chromophore, it must be borne in mind that there may be many different types of chromophore in one system, or that there may be different chromophores in different systems.

The most certain knowledge of the nature of the chromophore lies in the action spectra for photoreactivation (II-A-2). The only reasonably complete action spectra are those of Dulbecco (38) on T2 phage in *E. coli* B, Kelner (92) on *S. griseus* and *E. coli* B/r, Giese *et al.* (53) on *C. colpoda*, and Jagger and Latarjet (84) on *E. coli* B/r and T2 in B/r. Survival was the measured biological effect in all cases except that of *C. colpoda*, where division delay was the criterion. In all cases, incident radiation was measured. The only details in any of these spectra are the two small minima found by Jagger and Latarjet. Because absorption by *E. coli* above 3000 A is not accurately known, it is quite possible that these minima are caused by absorption by inactive cellular components, which screen some of the light from the PR chromophore. These four studies show that PR efficiency does not vary greatly within the range of effective wave lengths, a range that almost invariably falls within 3100 to 5000 A. One exception is *S. griseus*, which shows a single high maximum at 4358 A. The other organisms show small maxima around 3800 A, and *C. colpoda* shows in addition a small maximum at 4358 A.

These spectra are all rather disappointing in that they show (a) little significant detail, (b) little variation in efficiency, and (c) a rather narrow range, all of which makes identification of the chromophore extremely difficult.

It is likely that more than one chromophore exists, since some spectra show low efficiency where others show a maximum. As a working hypothesis, it is suggested that the common chromophore has an absorption maximum in the region of 3800 A, and that the two cases of high reactivation with 4358 A are a result of absorption by an efficient "alternate chromophore," with subsequent transfer of energy either to the primary chromophore molecule or to the reactivable site. The idea seems reasonable for *C. colpoda*, which shows equal maxima at 3800 and 4358 A. On the other hand, *S. griseus* shows only one high maximum at 4358 A. However, the effect at 3800 A in *S. griseus*, though far less than that at 4358 A, is still higher than that for *E. coli* at 3800 A, and in this case one need merely postulate that the alternate chromophore has an unusually high efficiency. Kelner (92) has suggested that this chromophore is a porphyrin. The spectrum of Giese *et al.* suggests riboflavin.

Jagger and Latarjet (84) have demonstrated that the action spectra for PR of a phage and of its host are identical (figure 2). This does not prove that the chromophore is identical in both cases, and therefore necessarily residing in the host. The chromophore could be nucleic acid, in one case that of the host, in the other that of the phage, which might be effective only after entry into the bacterium, when it presumably changes its physical state and/or contacts a reactive medium.

The PR spectrum is probably limited at both ends by factors that have nothing to do with absorption efficiency of the chromophore. At the lower wave length end, PR occurs with high efficiency in *E. coli* and its phage (figure 2) right down to the point where the light has a definite killing effect, which then masks reactivation below 3100 A. Therefore, proteins or nucleic acids could be the chromophores, even though they absorb so poorly above 3100 A, provided that PR is inefficient, in terms of incident photons per cell. At the high wave length end, the quantum energy above 5000 A may be too small to effect reactivation of damage caused by the 2537 A photon, which has twice as much energy (see IV-C-4). Consequently, almost any carotenoid, porphyrin, or similar molecule could be the chromophore, even though its principal absorption might be above 5000 A, provided again that PR is inefficient. Thus the possibility of there being a chromophore with maximum absorption outside the range 3100 to 5000 A depends on the efficiency of photoreactivation.

The efficiency of photoreactivation is not

known. There are two quantities that might be considered. One is the *cell quantum requirement* (inverse of cell quantum yield), or the number of photons, absorbed by the cell, required to photoreactivate the cell. The other is the *specific chromophore quantum requirement*, or the number of photons, absorbed by the PR chromophore(s), required to photoreactivate the cell. In studies of photosynthesis in algae, these two quantities are nearly the same, for a large fraction of the photons absorbed by the cell at the optimum wave length are absorbed by chromophores involved in photosynthesis. The two quantities are probably of the same order of magnitude for inactivation of *E. coli* at 2600 A, since here most of the photons are absorbed by nucleoprotein. In PR, however, it is not known what fraction of the photons is absorbed by chromophores that have nothing to do with PR. Photosynthesis requires about 10 visible photons absorbed per oxygen molecule produced (70). Inactivation of phage T2 requires about 3000 absorbed UV photons per phage (172), and inactivation of *E. coli* B requires about 10^6 absorbed UV photons per cell (80).

The data of Jagger and Latarjet (84) indicate that the ratio of (number of incident quanta at 3800 A to photoreactivate 50 per cent of the organisms)/(number of incident quanta at 2600 A to kill 50 per cent of the organisms) is about 400 for either the bacterium or the phage. Ratios of the same order of magnitude are indicated by the data of Giese *et al.* (56) for division delay in *Colpidium* and Christensen and Giese (30) for change in division pattern in *Tetrahymena*, although in these cases the ratio is difficult to calculate, for the effect studied is not all-or-none as in bacterial killing. Nevertheless, it is clear that several hundred times as many incident quanta are required for PR as are required for UV inactivation. Stein and Harm (147) showed the ratio of absorption at 2600 A to that at 3800 A to be about 7 for *E. coli* B (figure 7), and Jagger and Latarjet (84) showed about 5 for *E. coli* B/r. These measurements undoubtedly involve considerable light scattering, yet they indicate that the *cell* quantum requirement for PR is much higher than for inactivation, which shows that either (a) many substances unrelated to the reactivation are absorbing, or (b) the specific chromophore quantum requirement for PR is higher than for killing. Stein and Harm also showed that the 2600/3800 ratio for the absorption of DNA from *E. coli* B is about 140, whereas that for the bacterial RNA is about 1000. These measurements must involve much less light scattering than those for the bacterium, although some people feel that no nucleic acid "absorption" above 3100 A is real. The rough agreement of the ratio for DNA with that for killing versus PR, plus the fact that the DNA absorption remains fairly constant in the range 3200 to 5000 A, whereas that for RNA drops rapidly above 3400 A, suggests that DNA could be the chromophore for PR. If this were so, it would mean that the *specific chromophore* quantum requirement for PR was similar to that for inactivation, and it would also follow that much of the visible light is absorbed in substances unrelated to PR.

On the other hand, if the chromophore for PR were the same as for UV inactivation, it does not seem likely that both processes would have the same specific chromophore quantum requirement. The UV inactivates by producing damage anywhere in a certain target. However, the PR that follows is limited to the region damaged by UV, which is presumably only a small fraction of the original target.

These considerations are limited by ignorance of the true absorption of biological subjects in the range 3000 to 5000 A. In *E. coli*, for example, the absorption is quite low above 3000 A, and absorption measurements in an ordinary spectro-

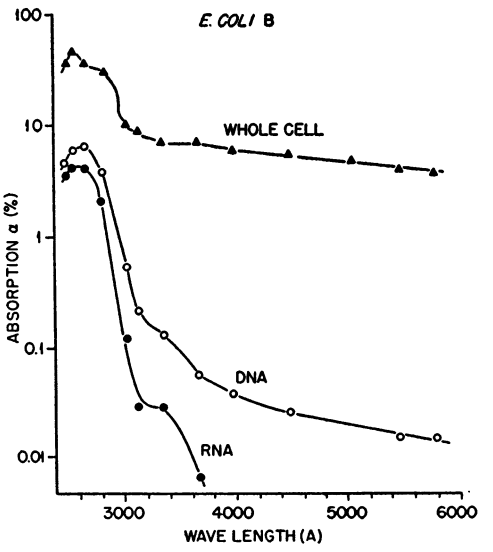

Figure 7. Absorption by the whole bacterium compared with absorption of its DNA and RNA. (From Stein and Harm (147).)

photometer are obscured by scattering and probably also by fluorescence (Jagger and Latarjet (84)). Other organisms, such as *S. griseus*, which shows higher absorption and better PR, might be easier to study in this respect than *E. coli*. It is important that such studies be made, for the interpretation of action spectra, especially those showing little variation of efficiency, requires knowledge of the absorption properties of the subject (Setlow (41)).

Several experiments outside the realm of action spectra have provided clues to the nature of the chromophore. The experiments of Skreb and Errera (145), cited earlier, show that PR occurs in enucleate amoebae. This indicates that something other than DNA is the chromophore for cytoplasmic PR, for it is generally accepted that amoebae contain little or no DNA in the cytoplasm. A similar argument applies to the work of Pierce and Giese (131), who found PR in enucleate nerve fibers containing little or no DNA. In the experiments of Blum *et al.* (17), with *A. punctulata*, a cytoplasmic red echinochrome pigment is present only in the enucleate half of the egg. Since PR is found in the nucleate half, the red echinochrome pigment cannot be the chromophore in the nucleate half. These studies therefore show that neither DNA nor this red echinochrome pigment is a *universal* chromophore but the studies do not show that these cannot be chromophores.

Bellamy and Germain (9) were unable to photoreactivate the bacteria, *S. faecalis* and *S. lactis*, under conditions that gave good reactivation in *E. coli* B/r and *A. aerogenes*. Since the first two lack iron porphyrin systems, the authors suggest that porphyrins are involved in PR. Goucher and Kocholaty (60) found that four strains of *Azotobacter* can be divided into two classes on the basis of their reflectance spectra, which are produced largely by cytochromes. The respiratory systems of one of these classes is less sensitive to UV inactivation and this class also shows little or no PR, whereas the other class has respiratory systems more sensitive to UV and shows good PR. Thus there is a good indication that, in these bacteria, PR is related to porphyrins. Latarjet and Beljanski (107), however, showed that, in two mutant strains derived from *E. coli* B and ML, containing less than $\frac{1}{500}$ the porphyrin of all types (measured chemically and spectroscopically) found in the parent strain, PR is nevertheless just as complete as in the parent strain. This shows that porphyrins are almost certainly not required for PR of *E. coli*, and normally have at most a small role, in agreement with the action spectrum. This finding lends some support to the suggestion made earlier that porphyrin may be an "alternate" chromophore.

Giese *et al.* (55) found no enhancement of PR by using a strain of yeast containing an excess of riboflavin. The result is inconclusive, partly for reasons given by the authors, and partly because the riboflavin content of the control cells might be quite high.

Examination of the action spectra for *E. coli* led G. H. Bowen (*personal communication*) to conclude that the most probable chromophores were pterins, flavins, or pyridoxal phosphate (pyridoxal itself having a similar but very weak absorption in this region) and he undertook experiments to distinguish among these. Careful measurements of the reaction rate constants for 3650, 4047, and 4358 A radiation in PR of phage T2 in *E. coli* B gave relative values for these wave lengths (corrected for quantum size) of 1, 1.08 $\pm$ 0.04, and 0.16 $\pm$ 0.01, respectively. In the same medium (pH 7), pyridoxal phosphate has relative extinction coefficients of 1, 1.04, and 0.176 at these wave lengths; no flavin or pterin has corresponding values at all similar. Bowen added to his system hydrosulfite, which would reduce flavins and probably pterins, but found no effect on PR, and thus concluded that the chromophore probably was not a pigment readily reduced to an inactive form, and in particular not a flavin or pterin. Pyridoxal is unaffected by hydrosulfite. From absolute measurements of dose rate and reaction rate at 3650 A, he also calculated the minimum molar extinction coefficient that the chromophore must have in order to account for the observed reaction rate with a quantum efficiency not greater than unity. This proved to be about 6000 to 6500 L mole^{-1} cm^{-1}, which at least does not exclude pyridoxal phosphate, for which the value is about 6500. Considerable evidence thus pointed to pyridoxal phosphate. Bowen therefore experimented with the addition to cells of 2×10^{-3} M pyridoxal. With cells grown in tryptone plus pyridoxal, then washed and infected with irradiated T2, there appeared to be a significantly higher rate of reactivation. When pyridoxal was added to the washed cells after

infection and was therefore present during illumination, there was a similar increased initial reactivation rate but this was quickly obscured by a rapid inactivation that was shown to be caused by a stable photoproduct (believed to be pyridoxic acid or its lactone) formed from the pyridoxal. This toxic product inactivates cells as colony formers, as well as destroying their capacity for producing phages. In both experiments, the increase in reactivation rate was small.

These excellent experiments of Bowen provide four lines of evidence pointing to pyridoxal or its phosphate as a chromophore for PR, yet the evidence does not prove that pyridoxal is indeed a chromophore. The experiments are of considerable interest, for they represent a detailed attack on the problem of identification of the chromophore and illustrate the elusiveness of this problem.

It is of interest that *S. faecalis*, which fails to show PR, is known to contain and require pyridoxal phosphate (111). This would seem to argue against this compound as a chromophore for PR. K. C. Atwood (*personal communication*), working with a pyridoxine-requiring strain of *Neurospora crassa*, found that conidia starved of pyridoxine, and presumably containing at least an order of magnitude less pyridoxine than the the controls, showed the same degree of PR as the controls. This is further evidence against pyridoxal or pyridoxal phosphate as a chromophore.

Many experiments have been performed in which PR *in vitro* of a particular molecule has been tested (IV-B). Although most of these attempts have failed, this does not eliminate the possibility that these molecules are chromophores in living systems.

The reactivable site (see next section) is nucleic acid in some cases. An economical assumption in these cases would be that nucleic acid is also the chromophore. The chromophore could be very nonspecific, such as strained nucleoprotein structures, perhaps with broken, but not displaced, hydrogen bonds. The similarity between PR and some other types of restoration that are nonspecific (see III-D) lends weight to this possibility.

Summary. Little is known of the location of chromophores for reactivation. They are known to exist in the cytoplasm but have not yet been shown to exist in the nucleus. They probably do, since in most cases the reactivable site is known to be in the nucleus. The nature of the chromophore is not yet known. It is almost certain that there is more than one, and it would not be surprising if there were a great many. The universality of photoreactivation suggests that the chromophores are fairly common molecules. There is evidence that DNA, porphyrin, riboflavin, or pyridoxal phosphate may be chromophores in some cases, although it is very unlikely that any of these is a universal chromophore. Proteins are not excluded, although RNA, because of its low absorption above 3400 A, probably is. The chromophore may be a nonspecific thing, such as weakened structure in a large molecule.

2. The reactivable site. The reactivable site is a crucial structure within the cell that suffers ultraviolet damage, directly or indirectly, that damage being photoreactivable (see IV-A). The reactivable site may or may not be identical with the chromophore or the chromophore molecule. It may be located far from the chromophore molecule. It may consist of one unit or many units, and these units need not be alike. It may be different in different organisms, and is probably different for different lesions. The location, the molecular nature, and the function of the reactivable site will be discussed here.

With regard to *location* of the reactivable site, it is to be noted that almost all photoreactivable effects involve reproduction, mutation, or transformation (II-B-2). These effects are caused primarily by damage to the nucleus, and therefore the reactivable site for most effects exists in the nucleus. The question then arises whether reactivable sites exist in the cytoplasm.

Blum *et al.* (17) found that delay of cleavage by ultraviolet in eggs of *Arbacia* occurs *only* when eggs or parts of eggs that contain either an egg nucleus or a sperm are irradiated, and in all these cases the damage is photoreactivable. Therefore, the site of this ultraviolet damage appears to be only in the nucleus. Cleavage delay not being a cytoplasmic effect, its study casts no light on the possible existence of reactivable sites in the cytoplasm.

In the experiments of Blum *et al.* (13) on *Arbacia* eggs (see II-B-2), the four nonphotoreactivable effects observed probably result primarily from nonnuclear damage. This sug-

gests that only nuclear damage is photoreactivable.

Von Borstel and Wolff (19) showed that hatchability of the egg of *Habrobracon* is inactivated when either cytoplasm or nucleus is irradiated, but that only the nuclear damage is photoreactivable. Thus sites affecting hatching in *Habrobracon* do exist in the cytoplasm, but they are not reactivable. It is interesting that, although the inactivation curves for cytoplasmic and nuclear damage differ, the action spectra are nearly the same and indicate nucleoprotein in both cases (3), which suggests similar chromophores but different mechanisms. This being so, it would appear that the reactivability of sites is to some extent a function of their location in the cell (Von Borstel (18)).

Brandt and Giese (22) found, in *Paramecium*, that division delay (which shows a nucleoprotein action spectrum and is presumably a nuclear effect) is photoreactivable, but immobilization (which has a protein action spectrum and is probably cytoplasmic) is not photoreactivable. This indicates that, in *Paramecium*, sites for immobilization exist in the cytoplasm but are not reactivable.

On the other hand, Skreb and Errera (145) found PR of survival in enucleate halves of amoebae. "Survival" in this case does not, of course, involve cell division. Pierce and Giese (131) found PR of decreased action potential and sensitivity of frog and crab nerves that contained no nuclei. These two experiments show clearly that reactivable sites exist in the cytoplasm, although they are the only experiments so far that show this.

As for the *molecular nature* of the reactivable site, the previous discussion indicates that, in most cases, it is something peculiarly nuclear, which immediately suggests DNA. The fact that mutation induction can be photoreactivated strongly suggests DNA. The existence of PR in phage almost requires that it be DNA, since the little protein of the phage that enters the bacterium does not appear to be important (69) and all of phage T2 nucleic acid is DNA (155). Finally, transforming factors, presumably pure DNA (81), have been photoreactivated (IV-B). Hence, it is certain that in some cases the reactivable site is DNA. The question that then arises is how universal DNA may be as a reactivable site.

Bawden and Kleczkowski (6, 7), working with plant viruses, which contain RNA but presumably no DNA (31), found PR of two spherical viruses (II-B-2). The effect appears to be clear-cut, although quite small. These experiments indicate that RNA is a reactivable site, but suggest that it is not so reactivable as DNA. PR of respiratory enzyme synthesis and of RNA synthesis (table 2) also suggest RNA.

The range of wave lengths that produce photoreactivable damage (II-A-1), as well as the existence of cytoplasmic PR and of PR of growth, suggests that the reactivable site can be either RNA or protein.

The *function* of the reactivable site is becoming evident from recent experiments. The UV sensitivities of several bacterial functions are listed in table 2. All these functions are photoreactivable. The distinction between "delay" and "cessation" is not clear in most cases since careful time studies usually have not been done. Most of these functions are inactivated exponentially, which implies that at all dose levels they are being inactivated, but at different rates.

Delay of cell division in *E. coli* B has been shown by Deering and Setlow (34) to occur at very low doses of UV. There is no inhibition of DNA, RNA, or protein synthesis at these doses, but the cells form long filaments. These filaments behave like single cells in protoplast formation, phage infection, and radiation inactivation (R. A. Deering, *personal communication*). After about 3 hr on nutrient agar, most of the filaments resume division.

Cessation of cell division in *E. coli* B occurs at considerably higher doses and is measured by decreased colony formation, the classical measure of radiation effect. At these higher doses, filaments are also formed but it is not known if any of them recover the ability to divide. The work of Magni (115) and Errera (47) indicates that PR of colony-forming ability affects only cells that otherwise could have formed filaments. Errera reported large numbers of paired nuclei in the filaments formed by 600 erg mm^{-2} of UV, although it is known that such doses considerably inhibit DNA synthesis. It seems likely that "killing" in *E. coli* B is caused primarily by effects on cell division.

In *E. coli* B/r, however, killing occurs only at higher doses, where DNA synthesis and other properties are affected, and filament formation

TABLE 2

Ultraviolet sensitivity of photoreactivable bacterial functions

Organism	Function	Delay	Cessation	Estimated Dose for 37% Survival* (erg mm^{-2})	Reference
Escherichia coli B	Cell division	×		4	
	Cell division (killing)		×	40	Deering and Setlow (34)
	Growth		×	200	
				2000	Errera (47)
Escherichia coli B/r	Cell division (killing)		×	1000	Witkin (171)
	DNA synthesis		×	1000	
	RNA synthesis		×	2000	Kelner (94); Iverson and Giese (82)
	Growth		×	2000	
	Respiratory enzyme synthesis		×	2000	Kelner (94)
Azotobacter	Respiratory enzyme synthesis		×	2000	Goucher *et al.* (61)

* These doses are in many cases estimated from data of the reference. They are to be construed only as representing an order of magnitude.

occurs only to a small degree or not at all (170), suggesting that effects on growth as well as on cell division are of importance.

Growth (increase in dry mass) appears in all cases to be less sensitive than killing (colony formation). In *E. coli* B, the estimates of sensitivity of growth differ by a factor of 10, but in either case UV killing is obviously not caused primarily by cessation of growth. Indeed, the very opposite has been suggested by Cohen and Barner (32), who found that other synthesis without DNA synthesis in *E. coli* leads to irreversible loss of the ability to multiply.

It is obvious that "killing" is an unsatisfactory criterion of UV damage to a bacterium. In an effort to get at the basic effect, Kelner (94) looked for properties of logarithmic phase cultures of *E. coli* B/r that were changed immediately after UV and by moderate doses, and that were reversible. Respiration, growth, and RNA synthesis were found not to be in this category, but DNA synthesis was immediately and completely stopped. Furthermore, PR caused immediate resumption of DNA synthesis. Further studies by Iverson and Giese (82) showed that, in out-of-log cultures, RNA as well as DNA synthesis was immediately inhibited by UV and both were photoreactivable. They postulate the existence of two sites of RNA synthesis, one in the cytoplasm and one in the nucleus, the cytoplasmic one predominating in logarithmic phase cultures and having a low sensitivity to UV compared with the nuclear one, which predominates in out-of-log cultures.

Kelner (95) has suggested that a common biological state, involving inhibition of DNA synthesis, exists in the cell after UV and eventually leads to the manifold end effects that are observed. He has suggested that PR be defined as "the phenomenon in which visible light removes the inhibition of DNA synthesis caused by UV light." Something along these lines may well be the true explanation of the UV effects, although, as Kelner himself has pointed out, the situation may not be so simple as the definition suggests.

Grundland *et al.* (62*a*), observing changes in nucleic acid content and electrophoretic mobility of *E. coli* after UV, suggest that PR is a repolymerization of UV-depolymerized nucleic acid. However, they did no PR experiments and hence have not tested this hypothesis.

There is considerable evidence that the genome itself is affected. The existence of PR of mutation and of phage induction points to the genome. Saracheck (140) has photoreactivated haploid, diploid, triploid, and tetraploid cells of *Saccharomyces*. For retardation of budding, the multiplicity of the inactivation curves follows

the ploidy, but PR reduces all the curves to exponential form, and therefore does not reduce the dose by a constant factor. This suggests that the photoreactivable damage is genetic, since it is related to ploidy, whereas the nonphotoreactivable damage is nongenetic, showing no relation to ploidy. Survival curves, on the other hand, show little relation to ploidy and PR gives rise to a constant dose-reduction factor (which is the same for all ploidies). These experiments suggest that, although the genome itself may be an important reactivable site, other sites are also important.

Kaplan (87) presents four arguments against the identity of UV inactivation and lethal mutation in *S. marcescens*, (a) mutations are always one-hit, whereas killing multiplicity varies with conditions, (b) the photoreactivable sector for mutation is larger than for killing (0.90 versus 0.72), (c) with holding time at 26 C in saline, photoreactivability of mutation is lost faster than photoreactivability of killing, (d) doses of long ultraviolet (3100 to 4000 A) that do not affect survival can induce mutation to the same degree as UV (2537 A) that inactivates to 15 per cent survival. Zelle *et al.* (174) showed that the photoreactivable sector for mutation in *E. coli* SD-4 is considerably larger than that for killing (0.82 versus 0.54).

It is evident that UV produces two types of damage, one photoreactivable and the other not. Whether these damages differ in kind rather than simply in degree is not known. The largest photoreactivable sector for killing occurs in phage T1 (0.68), and the largest sector for mutation has been found in *S. marcescens* (0.90). Nishiwaki (126) working with *E. coli* communior, estimated the size of the photoreactivable target for killing as equivalent to a sphere of about 70 A diameter, with a slightly smaller target size for a streptomycin-resistant strain. It will be recalled that, in *E. coli*, the action spectrum for the photoreactivable and the nonphotoreactivable sectors is the same (see II-A-1), and in both cases indicates involvement of nucleic acid or nucleoprotein. If the chromophore and the reactivable site are the same, this indicates that both types of damage occur in the same type of molecule.

Many things other than the genome could be reactivable sites and there is little evidence to eliminate possible sites. The fact that enucleate cells can be reactivated (see above) strongly suggests reactivation of other things than DNA or sites of DNA synthesis.

Summary. The reactivable site is usually in the nucleus but it can be in the cytoplasm. In some cases it is known to be DNA and in others it is probably RNA. Other possibilities have not been eliminated. Functions of reactivable sites include (in bacteria) cell division, nucleic acid and enzyme synthesis, and growth. These could all be controlled by nucleic acid and some evidence points to the genome. However, it is likely that there are other important reactivable sites. Not all of the ultraviolet damage is reactivable.

B. In vitro Experiments

Reference has been made to various experiments on photoreactivation outside the living cell. Only one of these has met with unqualified success. Nevertheless, a discussion of all such attempts is worthwhile, for this type of experiment will eventually contribute greatly to an understanding of photoreactivation.

There are two examples of restoration that are not photoreactivations but are nevertheless pertinent to the subject. In one of these, ultraviolet damage is restored chemically and thermally. In the other, chemical damage is restored with light. Both are purely chemical systems.

Sinsheimer (144) showed that very long exposures of uridylic acid to UV radiation causes complete loss of the characteristic pyrimidine absorption peak at 2600 A. Subsequent treatment at pH 0.8 for 20 hr, or by holding at 85 C for 4.5 hr, causes 90 to 100 per cent restoration of the pyrimidine peak, and the molecule appears to have regained all its original physicochemical properties. Further studies by Wierzchowski and Shugar (169) showed that cytosine and 1-methylcytosine belong to a class in which UV causes a great loss in absorption at 2700 A, and that this damage is irreversible at high doses. Cytosine nucleosides and nucleotides, however, belong to a class which shows a smaller loss in absorption at 2700 A with the appearance of a new maximum in the region of 2300 A, which changes are reversible even after high doses of UV and also to some extent at neutral pH (*i.e.*, spontaneous reversal). Cytosine and uracil and their derivatives are much more sensitive to UV than purines or other pyrimidines, and they are also the only two that show heat and acid reversibility.

The work of Moore and Thomson (120) on

1,3-dimethyluracil showed that the inactivation observed by Sinsheimer probably involved the addition of water to the 5,6 carbons of the pyrimidine (OH at the 6 position), with consequent disappearance of the double bond at this point and loss of the 2600 A absorption peak. Acid or heat treatment removes this water molecule, restoring the double bond and characteristic absorption. Wierzchowski and Shugar (169) showed that this mechanism is probably correct for all the above cases and further that reversibility among the nucleosides and nucleotides depends upon the existence of hydrogen bonding between (principally) the 5′ sugar hydroxyl and the pyrimidine carboxyl (opposite to the 5,6 bond), which makes the 5,6 bond more susceptible to the addition of water. In addition, the 5,6 carbons must be unsubstituted. Shugar and Wierzchowski (143) have found indications that UV alteration of nucleic acids is also reversible with acid or heat treatment. These experiments are of great interest, for they concern the only case in which is known exactly what happens in the UV inactivation and subsequent restoration of a biomolecule.

Shugar (142) performed an experiment which is a reactivation with light of chemical damage. Crystalline triosephosphate dehydrogenase, which is firmly combined with diphosphopyridine nucleotide (DPN), is partially inactivated during preparation owing to oxidation of essential SH groups. Shugar found that it can be restored with near UV light, and that the maximum in the action spectrum is at 3400 A, corresponding to the absorption peak of reduced DPN. Probably what happens is that, during inactivation, the SH groups are oxidized to S—S, with consequent reduction of DPN to DPNH. The latter can then absorb light, which provides it with energy for reversing the reaction. The restoration is approximately a first-order reaction, as in the case of PR of phage T2 (III-A). Also, the spectral range for reactivation is rather similar to that for photoreactivation. This experiment is of importance because it shows that this particular type of damage can be restored by light.

Attempts have been made to photoreactivate *biomolecules in a simple medium*. Results are variable. Wells and Johnson (167) used adenosine triphosphate (ATP) in water at pH 7. No PR was found, as measured by change in absorption at 2600 A. Intense irradiation with visible light alone did not alter the absorption at 2600 A but rendered the ATP an unsuitable substrate for rat liver ATPase. B. Ekert and J. Jagger (*unpublished data*) attempted PR in phosphate buffer of the UV-induced loss of absorption at 2600 A, using both uridylic acid b and 1,3-dimethyluracil, as well as several analogues of these compounds. No effect was found. Extremely high doses of visible light after UV tended to cause further loss of absorption.

Wells (164) reported a true photoreactivation of DPN. In his experiment, solutions of DPN in 0.1 M phosphate buffer were exposed to UV or to UV followed by visible light. These solutions were then added to methylene blue solutions in the caps of Thunberg tubes containing hog- or rat-liver homogenates in phosphate buffer. The tubes were evacuated and the solutions mixed. The process measured is evidently the ability of DPN to accelerate hydrogen-transfer reactions, which decolor the methylene blue. This ability is reduced by UV irradiation of the DPN in phosphate buffer and is photoreactivated by visible irradiation of the DPN in phosphate buffer. It is therefore a real *in vitro* photoreactivation. The experiment is not highly satisfactory, however, since (a) it uses a chemically complex assay (offering the possibility of alternate metabolic pathways), (b) the four experiments reported show considerable variability, and (c) the DPN is, on the average, only inactivated to 60 per cent of its normal activity and then photoreactivated up to 75 per cent activity. The observed effect, although always positive, is really quite small.

Ekert and Monier (44) have attempted, unsuccessfully, to repeat Wells' experiment. They used two different types of assay based upon appearance of the band at 3400 A in reduced DPN, both of which gave highly reproducible results, and they used the assay of Wells, which did not give good reproducibility. None of the three assays revealed any PR. Ekert and Monier were unable (a) to find any PR of DPN *in vitro*, (b) to find any evidence of reduction of DPN by UV, or (c) to account for the observation of Wells, even if it were not a PR phenomenon. Their study was more complete than that of Wells and one can only assume, pending further experiments, that PR of DPN *in vitro* has not yet been demonstrated.

The experiments discussed so far show that

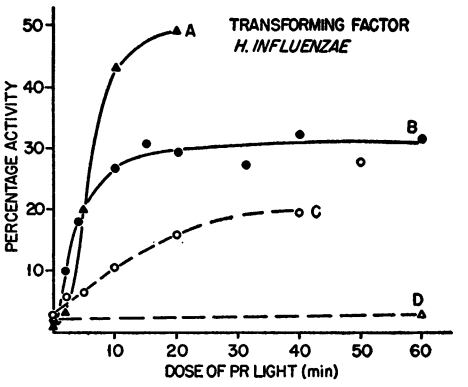

Figure 8. Transforming activity of a transforming factor (DNA) for streptomycin resistance during photoreactivation at 37 C, expressed as per cent of uninactivated controls. Reaction mixtures contained 4 vol of UV-inactivated DNA, 1 vol of M/10 $MgSO_4$, and the following volumes of *E. coli* B extract: (*A*) 1 vol of the most active preparation of extract, incubated light, (*B*) 2 vol of the same extract preparation after 6 weeks' storage at −20 C, incubated light, (*C*) 1 vol of an extract preparation having more typical activity, incubated light, (*D*) 1 vol of the same extract, incubated dark. Light was provided by a tungsten projection lamp. Control mixtures were identical with corresponding reaction mixtures except that the DNA was not exposed to ultraviolet. (From Rupert *et al.* (139).)

no PR has been clearly demonstrated using biomolecules in simple media. Other experiments have been done with *biomolecules in a complex medium.* For molecules that do not carry genetic information, one experiment shows a small effect. C. W. Shuster and J. L. Larimer (*personal communication*), using a crude extract of mouse brain cells, found an apparent reactivation with visible light of triosephosphate dehydrogenase, as measured by reduction of DPN. This experiment, however, involves effects so small that they are not known to be true PR.

Of "molecules" that carry genetic information, phage shows no effect but transforming factor shows an outstanding effect. Dulbecco (38, 40) showed that phage T2 could not be photoreactivated before irreversible adsorption to the host. He tried to get phage PR under the following conditions: (a) phage alone in liquid suspension, (b) phage alone on nutrient agar, (c) phage in a suspension of bacteria lacking NaCl (to prevent adsorption), (d) illumination of bacteria in solution for long periods up to the moment of addition of phage, (e) the same on nutrient agar, (f) in the presence of bacteria killed by heating to 60 C for 20 min, and (g) in the presence of cell-free bacterial extracts, produced either by grinding frozen cells or by ultrasonic disruption. Subsequent experiments by Bowen (see III-B) showed that injection of the phage DNA into the host cells was necessary before PR could occur. These experiments indicate that it is very unlikely that there is phage PR outside a living cell.[13]

Goodgal *et al.* (58) reported PR of a transforming factor *in vitro*. They found that *H. influenzae* is not photoreactivable under conditions giving good PR in *E. coli*. Also, the transforming factor for streptomycin resistance of *H. influenzae* is not photoreactivable either in this bacterium or in extracts, but it is photoreactivable in extracts of *E. coli* B. Subsequent experiments by Rupert *et al.* (139) have clearly demonstrated that, in this case, *in vitro* PR does indeed exist. Transforming factor inactivated to about one per cent survival can be reactivated in this manner to 50 per cent survival, although reactivation to about 20 per cent survival is more typical (figure 8). The PR stops immediately upon extinction of the light and resumes immediately upon illumination. There is a definite dose rate effect, only low rates permitting reciprocity of time and dose rate. This curve is similar to that found by Dulbecco for phage T2 (figure 6). There is a definite temperature effect, showing a Q_{10} that agrees well with *in vivo* results (table 1).

The inactivated transforming factor lost no activity in 20 hr at 37 C or in 11 weeks at −20 C. It could be heated to 90 C for one minute with only a minor loss of transforming activity or of photoreactivability.

Rupert *et al.* have investigated the nature of the extract quite carefully. Cells of *E. coli* B entering the stationary phase were ruptured by grinding, by ultrasound, or by pressure on a mass of frozen cells. The broken-cell suspensions were centrifuged at 10,000 G for 40 min, and experiments have shown that the cells remaining in the supernatant do not contribute to its photo-

[13] An exception might appear to be the PR of phage in UV-killed bacteria (40). But these "killed" bacteria have lost little but the ability to divide; they are still growing, metabolizing cells, capable of supporting growth of normal phage.

reactivating power. Cells in the logarithmic phase of growth gave distinctly inferior extracts. Results with different extracts at any one phase were, however, highly variable. Intact viable cells produced no PR of the transforming factor. Magnesium ion was found to be often necessary to prevent inactivation by the extract. Addition of ATP has no effect, nor has preillumination of the extract.

The active agents in the extracts were found to be at the molecular level. Sedimentation at 110,000 G for 1 hr causes no loss of extract power. Dialysis has separated the extract into two parts, both of which are required for PR, and the system can be reconstituted after dialysis (figure 9). The dialyzable fraction contains a component that limits the maximum degree of reactivation, but not the rate of reactivation. The nondialyzable fraction is inactivated by 90 C for 1 min, while the dialyzable fraction is little affected by this treatment.

R. Latarjet (*personal communication*), working with *D. pneumoniae*, found no PR of a transforming factor for streptomycin resistance, either when illuminated alone or inside its host, nor did he find PR of the host itself. R. Latarjet and N. Rebeyrotte (*personal communication*), however, found PR of the transforming factor when it was illuminated in an extract of *E. coli*. Although the reactivation they found was small, it was nevertheless significant.

There appears to be no question that PR *in vitro* has indeed been found. The experiments indicate that PR requires a particular chemical system, which does not occur in all bacteria. The system includes a large molecular weight fraction that is heat labile, and a small molecular weight fraction that is heat stable and whose concentration limits the maximum degree, but not the rate, of PR. It seems likely that the large fraction contains an enzyme and the small one a cofactor that may be irreversibly used up during the reaction. The dose rate effects suggest that some substance is destroyed by the light, but regenerated in the dark, and that the concentration of this substance limits the rate of reactivation; it appears, therefore, that this substance is not the presumed cofactor.

The apparent complexity of the system required for PR may explain earlier failures to find *in vitro* PR where a simple medium was used. The failure to find *in vitro* phage PR suggests that the protein coat of the phage shields it from the necessary chemical interaction.

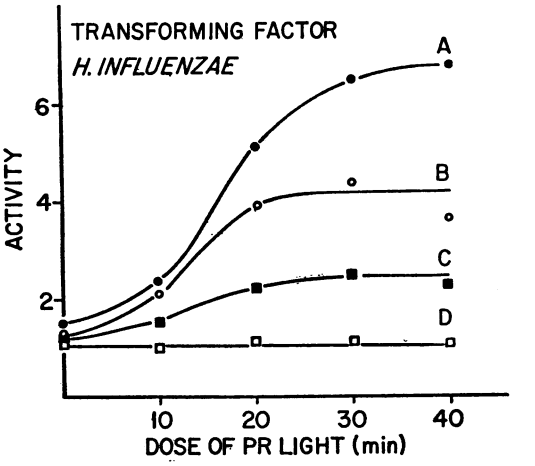

Figure 9. Photoreactivation of a transforming factor (DNA) for streptomycin resistance with different dilutions of concentrated dialyzate. Activity in hundreds of transformed cells per ml. Reaction mixtures contained 4 vol of UV-inactivated DNA, 1 vol of M/10 $MgSO_4$, 1 vol of dialyzed *E. coli* B extract, and 1 vol of concentrated dialyzate at the following dilutions: (*A*) full strength dialyzate, (*B*) 2 × diluted, (*C*) 4 × diluted, (*D*) infinitely diluted. (From Rupert *et al.* (139).)

Summary. Photoreactivation of biomolecules in a simple medium has in no case been clearly demonstrated. Systems used include ATP in water, uridylic acid and analogues in phosphate buffer, and DPN in phosphate buffer. Of experiments with biomolecules in a complex medium (cell extracts), one experiment with enzymes shows a small effect, phage shows no effect, but transforming factor shows a very definite effect. A transforming factor of *H. influenzae* is photoreactivated in *E. coli* extracts. The essential components of the extract are soluble and can be separated into two parts, one dialyzable and heat stable, the other nondialyzable and heat labile.

C. Theoretical Models

What will be attempted here is a summary and discussion of the most important models and hypotheses that have been suggested for photoreactivation. For convenience of treatment, these have been divided into four categories representing a transition from the most complex and in-

direct mechanisms to the simplest and most direct ones.

A point has now been reached in this treatment at which the word "photoreactivation" itself no longer suffices and more detailed terminology must be established. It seems convenient to hinge definitions on one important point in the chain of inactivation events, the inactivation of the reactivable site. The definition of the reactivable site has been discussed (IV-A). The term "restoration" refers to any treatment applied after the start of irradiation which, in any way whatsoever, undoes the damage to the *cell* caused by inactivation of the reactivable site. This damage may be undone in three ways: the reactivable site may be *by-passed*, implying that the inactivated site is not changed by the restoration; damage to the reactivable site may be *prevented*, implying that the chain of inactivation events is broken before the reactivable site is affected; or damage to the reactivable site is *reversed*, implying that the damage to the *reactivable site* is undone or that the reactivable site is "reactivated." Thus, "by-pass," "prevention," and "reversal" are all microscopic aspects of the macroscopically observed "restoration" of cell damage.

1. Alternate pathways. It is most important to establish whether photoreactivation really involves a prevention or reversal of the ultraviolet damage. If, instead, the reactivated cell utilizes alternate pathways, then it should behave somewhat differently from unreactivated cells at the same survival level.

The only such changes after inactivation and reactivation that have been carefully investigated concern UV sensitivity. Lennox *et al.* (110) showed that complexes of *E. coli* B and phage T2 show the same UV inactivation curves after PR as unreactivated complexes at the same survival level. Similar results were obtained with *E. coli* B and phage T7. These experiments indicate that, for bacterium-phage complexes, the damage has not been by-passed, but has been prevented or reversed, at least insofar as UV sensitivity is a measure of this.

Dulbecco (40) quotes Novick as having done a similar experiment, in which *E. coli* B/r was inactivated with UV three times, with two alternate photoreactivations. The third UV inactivation gave a survival curve the same as that obtained the first time. Newcombe and Whitehead (123), working with *E. coli* B/r grown in synthetic medium, found that the percentage *mutation* (color response on mannitol-tetrazolium agar) was induced and reversed to the same degree after three UV doses alternating with two visible light doses. After the first cycle of UV and visible, however, *survival* of the bacteria showed greater sensitivity to further UV, and further visible light was lethal. It is unfortunate that Novick's results are not published, permitting a comparison of techniques. The lethal effects of light are well known (III-C), and increase rapidly at lower wave lengths. It is possible that the source used by Newcombe and Whitehead (B-H6 with filters) passed wave lengths much shorter than that used by Novick. With *E. coli* communis, Nishiwaki (125) showed that the *fourth* application of UV, after three photoreactivations, showed the same survival curve as with no PR (figure 10).

These experiments show that, for survival of bacterium-phage complexes, for bacterial mutation, and in some cases for bacterial survival, PR does not change UV sensitivity. This suggests that the UV damage has indeed been prevented or reversed, and not simply by-passed. Of course, these experiments do not prove that reactivated cells are exactly like unreactivated cells at the same survival level, for even though the UV sensitivity is unchanged, other properties may have changed.

This possibility is suggested by the experiments of Pittenger and McCoy (132) on spores of *S. griseus*, in which five UV exposures (which would normally give 10^{-5} survival), alternating with PR, gave 30 per cent survival and a percentage mutation far higher than would be obtained with a single inactivation to 30 per cent survival. In this case, therefore, the photoreactivated organisms are different, although this difference is probably slight, since so many cycles were required for a large effect. Newcombe and Whitehead (123) did similar experiments with *E. coli* B/r and found no effect, but they used only 1½ cycles. They did find, however, that mutation after high doses of UV was not photoreactivable, and they, as well as others have found different dose reduction factors for killing and mutation. There is thus some evidence that photoreactivated organisms are not identical with nonphotoreactivated organisms at the same survival level.

It has been shown (83) that PR of induction of lysogenic *P. pyocyanea* leaves surviving clones that are all lysogenic and carry the original prophage, strongly suggesting that the UV damage is not by-passed.

The UV damage to bacterial transforming factor is certainly not by-passed in photoreactivation (IV-B), since the only substance that enters the host cell after reactivation is the DNA itself, and there does not appear to be any multiplicity reactivation.

There is no direct evidence on this matter for organisms higher than bacteria. But the very existence of PR of mutation in many such organisms suggests that in all cases at least part of the ultraviolet damage is prevented or reversed, and not by-passed.

Summary. There is limited evidence that photoreactivation in bacterial systems (including phage and transforming factor) does not operate primarily through enhancement of alternate pathways but that it represents a prevention or reversal of the ultraviolet damage itself. This conclusion probably applies also to higher organisms.

2. Neutralization of poisons. Photoreactivation very likely operates by prevention or reversal of the ultraviolet damage rather than in by-passing this damage. "Reversal" is herein treated as being either direct or indirect reversal, and "prevention" as the neutralization of poisons. In the broadest sense, poison mechanisms would include reversal mechanisms, the reactivable site itself being considered a cell poison. However, such extremes do not seem to add to understanding and consequently in this review poisons will be considered only in the classical sense of agents that are produced at some site other than the reactivable site and that then migrate to the reactivable site and inactivate it.

If it is assumed that (a) the amount of poison produced is directly proportional to the radiation dose, (b) the amount of poison decaying at any time is proportional to the poison concentration at that time, and (c) the rate of inactivation by the poison is proportional to its concentration, then it can be shown that exponential survival will not be observed when the decay time is of the order of magnitude of the irradiation time. Furthermore, reciprocity of time and dose rate will not be observed if the range of times includes the decay time of the poison.

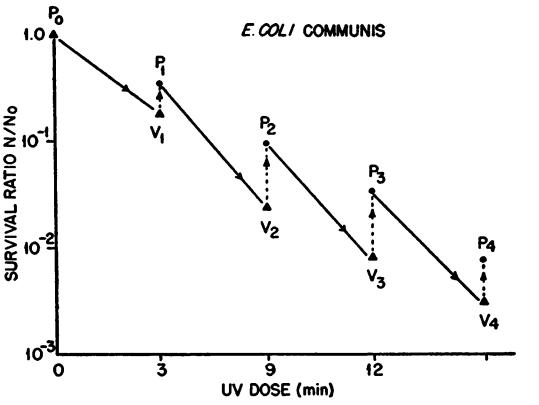

Figure 10. Four cycles of ultraviolet inactivation and photoreactivation in a bacterium. The slopes of the ultraviolet curves do not differ significantly (Nishiwaki (125)).

A poison may decay because of (a) spontaneous breakdown, (b) interaction with a reactivable site, (c) interaction with other sites, or (d) such factors as diffusion out of the cell, dilution by cell growth or cell division, or change in the state of the cell such that the poison is no longer effective. All these modes of decay may be grouped together, for they can all be considered to reduce the amount of poison at a rate proportional to the concentration.

Loss of photoreactivability with time occurs in metabolizing systems (III-B). The data for bacteria indicate that, if poisons are involved, their decay time is not greater than about 4 hr at 37 C. This elimates poisons with very long decay times.

Reciprocity of time and dose rate has been found to hold at metabolic temperatures for UV exposures from a few microseconds to 10 sec for protozoa (133) and to about 1 hr for *E. coli* (134). Rentschler *et al.* (134), using *E. coli* growing on nutrient agar plates, showed that fractionated doses, ranging from 16 exposures at 1-hr intervals to continuous exposure for 16 hr, gave reciprocity. Koller (103) showed reciprocity with *E. coli* communis at room temperature for exposures from 2 sec to 2 hr, and at 0 C for exposures from 3 min to 18 hr. Reciprocity experiments thus tend to eliminate poisons with intermediate decay times.

Exponential survival with UV is often observed. This is inconsistent with the idea of a poison that decays in a few minutes.

The remaining alternative, of poisons with

microsecond decay times, can be discarded, since PR light, which will always work after UV, would not have time to act upon so transient a poison.

These experiments therefore tend to eliminate the possibility of poison mechanisms in PR. This argument, however, should not be taken too seriously, because it is based upon ideal mechanisms, which are undoubtedly oversimplified. It may be noted that experiments to test reciprocity or temperature effects over long periods of time obviously encounter interference from metabolism. Studies with nonmetabolizing organisms indicate the presence or absence of independent poisons, but not of poisons that require metabolism for their action.

Temperature effects are notably absent during UV inactivation, the Q_{10}'s usually being close to 1, whereas the Q_{10}'s observed for most poisons are in the range 2 to 8. This suggests that poisons that act in a time of the same order as that of the irradiation (usually a few minutes) are not produced.

Direct observation of irradiated organisms indicates drastic immediate effects at normal doses (133),[14] and even with low doses effects are observable within an hour and no new inactivation effects appear after this time (IV-A-2). These observations argue against the existence of slow-acting poisons.

The fact that normal PR is usually observed even when the UV has been administered to a subject in the *solid state* (II-B-2) argues against poison mechanisms. This applies particularly to phage and transforming factor, where only the irradiated DNA enters the bacterium. The fact that the cross sections for inactivation of intracellular and extracellular phage are similar (114) suggests that the mechanism is similar in both cases and hence does not utilize cellular poisons.

A *constant dose reduction factor* (constant DRF) has often been interpreted to imply the operation of a poison, presumably as contrasted with a direct effect. In the target theory, the final slopes of survival curves are proportional to the size of individual targets. If the number of hits or targets differs, with the same target size, these slopes will be parallel and will not extrapolate back to the same point on the ordinate. Constant DRF curves, on the other hand, are not parallel and extrapolate back to the same point on the ordinate (figure 4). Thus, constant DRF curves imply that PR has not changed the *number* of hits or targets involved but rather that it has lowered the effective dose before it reaches these targets. However, if PR should operate by changing the *size* of individual targets, without changing the number of hits or targets, then a constant DRF would also be observed. In this case, instead of decreasing the effective UV dose, PR is decreasing the final sensitivity of the individual targets, which produces the same net effect of constant dose reduction. These targets may be considered to be specific structures. Therefore, a constant DRF does not necessarily imply a poison mechanism in the classical sense.

Furthermore, a constant DRF appears to be the exception and not the rule (III-A), although the deviations from constancy are not so great as to preclude its great usefulness as a qualitative concept. There are a few clear cases of constant DRF. The first was reported by Kelner (90) for *E. coli* B/r. This was soon repeated by Novick and Szilard (128), who were careful to point out that they were invoking a poison theory only for the sake of argument. They observed a constant DRF for both survival and mutation. Warshaw (157) observed a constant DRF with both haploid and diploid yeast, and attributed this to a poison effect. Northrop (127), working with *B. megatherium*, also postulated a poison theory. He assumed that the inactivation is not photochemical, since cultures kept at 15 C in the dark retain their photoreactivability much longer than those kept at 35 C. This, however, merely shows that the UV damage changes its form with time and temperature. Northrop did not study effects where a constant DRF could be observed.

Dulbecco (39) showed that the simple genetic recombination theory of multiplicity reactivation was incompatible with observed survival curves for phage T2 in *E. coli* B, and that PR decreased the apparent hit number (extrapolate to ordinate), thus failing to show a constant dose reduction factor. Barricelli (5) has interpreted

[14] Kelner (94–96) has reported that, after UV irradiation of *E. coli* B and B/r and *Bacillus mycoides* in the exponential growth phase, the growth rate (turbidity increase) drops within a few seconds, and then *remains exponential* for about an hour. This suggests that UV damage to the growth mechanisms occurs immediately and is completed in a few seconds.

this in terms of a formal theory that supposes UV damage to occur not only in ordinary genes but in certain "vulnerable centers" (which could be "key" genes for virus replication) that represent a considerable fraction of the total target. UV inactivates three such centers in T2 and two of these are completely photoreactivated. PR of both the ordinary genes and the vulnerable centers effectively reduces not only the number of targets inactivated, but also the size of the remaining targets.

Brandt *et al.* (23) conducted an interesting and clever experiment on the carnivorous ciliate *Didinium nasutum*, which feeds on paramecia. UV inactivated paramecia were fed to *Didinium*, whereupon the carnivore grew at the same rate as on normal paramecia, and divided at the same rate or faster. Paramecia treated with hydrogen peroxide, however, caused a decrease in division rate of *Didinium*. This suggests that poisons that can survive digestive processes (presumably including organic peroxides) are not produced to any great extent by UV.

Summary. It is unlikely that poisons of the classical type are acted upon in photoreactivation of bacteria. Extremely fast-acting (microseconds) poisons may be produced by ultraviolet, but photoreactivation could not operate on these before they had reacted. In a few cases, it is certain that poisons of the classical type are not involved. Therefore, photoreactivation appears to operate primarily by reversal, rather than prevention, of the ultraviolet damage, although in some systems poisons could be important.

3. Indirect reversal. Mechanisms of photoreactivation involving reversal of the damage to the reactivable site may be classed as "direct reversal" or "indirect reversal" according to whether or not the reactivating photon is absorbed by the reactivable site.

It is apparent that the question of the mechanism of photoreactivation depends to a great extent on the mechanism of ultraviolet inactivation. Little is known about either. These problems are twins and their solutions will probably advance in parallel fashion. Consequently, speculation about the nature of photoreactivation involves speculation about the nature of ultraviolet action.

An almost total lack of dependence on environmental factors (Giese (52)) suggests that UV action consists in large part of a relatively direct effect on an important molecule, which may be considered the reactivable site. The fact that some treatments may protect or restore from as much as 90 per cent of the UV effect does not eliminate direct action as a mechanism (85). This would mean that the chromophore for inactivation was the reactivable site. The chromophore is known to be nucleoprotein in the bacteria (173) as well as in many other organisms. Also, the reactivable site is probably either DNA or RNA in many cases (IV-A-2). There is consequently a good case for the idea that UV inactivation consists primarily of a direct action on nucleic acid or nucleoprotein and that the latter is also the reactivable site for photoreactivation.

Photoreactivation, on the other hand, is strongly dependent on environment (III-C). This, as well as the fact that PR does not occur in all organisms (II-B-2) (which all contain essential nucleic acid), tends to make indirect reversal a somewhat more favorable hypothesis than direct reversal.

If indirect reversal is the most likely mechanism for photoreactivation, it is probably worth while to see how much can be said about its details, even to the point of indulgence in mild speculation. The chromophore is probably not the same kind of molecule as the reactivable site. Consequently, of the likely chromophores (see IV-A-1), nucleic acid can be eliminated, leaving protein or substances, such as porphyrin or flavin, which absorb efficiently above 3000 A. It is difficult to make a choice between protein and other more efficient chromophores, for proteins may very well be produced directly by DNA (26), and would thus possess the specificity for DNA repair. It is economical to assume that the chromophore is spatially not far from the reactivable site. In the system of Rupert *et al.*, (139) it is known that at least two substances are required for PR, in addition to the reactivable site. It seems likely that one of these is protein, perhaps an enzyme, and the other a cofactor. This cofactor, however, is not the substance that is regenerated in the dark (IV-B). That substance could be Rupert's protein, or the chromophore, or another substance. Rupert's protein could be the chromophore.

Thus one can draw a picture, based partly on experimental data and partly on speculation, of the most likely mechanism of PR. It appears

that the UV is absorbed in nucleic acid that is associated with DNA synthesis and that this absorption entails cessation of that synthesis. Visible light is then absorbed in a chromophore, possibly protein, and, in an enzymatic reaction involving a cofactor, reverses the UV damage to the nucleic acid, with consequent resumption of DNA synthesis. This picture is consistent with most of the experimental data, but most certainly is not required by them. In particular, it is based largely on information obtained with bacterial systems.

It is of interest, and a tribute to the vision of the early experimenters in this field, that the basic outline of this picture was suggested in Dulbecco's review, written only three years after the discovery of photoreactivation.

Summary. Indirect reversal seems the most likely mechanism for photoreactivation. A somewhat detailed picture of this mechanism may be drawn, based partly on experimental findings and partly upon speculation.

4. Direct reversal. A mechanism of direct reversal is attractive because it is simple. Several workers have considered this form of mechanism and present knowledge of photoreactivation has by no means eliminated it, at least as an important contributing factor. (Of interest is the discussion by Errera (46) of activation of enzymatic systems by light.)

That such a mechanism is possible with UV and visible wave lengths, and that it may operate in strange ways, is illustrated by the experiments of Hirshberg (75). He found that certain spiropyrans, with no absorption above 4000 A, when irradiated with 3650 A in polar organic solvents at low temperatures (about 180 K), became colored, showing intense absorption in the visible range, with distinct maxima. Subsequent irradiation with visible light caused a total loss of color. The curious thing is that, in one case, the most effective reversion was obtained by irradiating at a visible wave length (4358 A) where the colored compound showed practically no absorption, whereas the least effective wave length tried was that at the absorption maximum (5460 A). The other compounds showed similar but not such striking effects. Hirshberg postulates that reversion requires a quantum of a certain minimum energy, quanta of lower energy producing essentially no effect even when they are more efficiently absorbed. He suggests that the mechanism involves opening of the C—O bond in the pyran ring by the UV. The exposed oxygen acquires a hydrogen ion from the medium if the medium is polar, and this effect is greatly accelerated in acid solution. Visible light can then reclose the ring, with loss of the hydrogen ion. These experiments, of course, were conducted at low temperatures in special media. Nevertheless, they show that a PR mechanism of direct reversal is possible.

The experiments of Sinsheimer and others on heat and acid restoration of nucleic acid components, previously discussed (IV-B), may well have direct significance for PR, although the systems involved have not been shown to be photoreactivable.

Stein (146) discusses the general case of radiation-produced metastable states in biological systems, pointing out that the work of Hirshberg, as well as that of many others, provides a possible mechanism for PR. He also mentions the similar case of *cis-trans* isomerization, as well as electron capture in crystals. Since much of his discussion concerns phenomena other than PR, his paper will not be discussed in detail.

Duchesne and Garsou (37) have considered the work of Hirshberg, Stein, and others, and present the most recent discussion of possible direct reversal mechanisms in PR. They consider the two most important possibilities to be (a) metastable states in large molecules, and (b) internal molecular ionization with subsequent capture of the free electron in structural faults. In the second mechanism, reactivation involves removal of the captured electron from the fault and return of it (or another electron) to the original position. However, such a mechanism is very likely to be affected by oxygen, which could carry off the trapped electrons. Since no oxygen effect has been observed in PR, the authors dismiss this mechanism as less likely than that of metastable states; (however, note result of Sbarra and Hollaender, III-C). They propose DNA as the important molecule, not only because of its biological importance, but because it shows a highly ordered structure, a low coefficient of thermal expansion, and high thermal (and hence probably electrical) conductivity, all of which would favor formation of metastable states of long life, and eventually of structural faults. Inactivation would entail displacement of excited electrons toward the center of the molecule where

oxygen and nitrogen atoms, because of their high electronegativity, would tend to retain the electrons, forming a heteropolar molecule. This mechanism would also entail the opening of aromatic rings and should not show an effect of extramolecular oxygen, but should show a positive temperature coefficient, in agreement with experiments on PR. Finally, the authors suggest that such a sustem could also account for production of mutations.

Some arguments against direct reversal come from experiments on PR. The lack of instances of PR in a truly dry state (see II-B-2), renders unlikely a purely direct effect. The failure of *in vitro* experiments in simple solvents (IV-B) as well as the difficulty of obtaining PR even in cell extracts also argue against a direct effect.

Summary. Direct reversal is theoretically an entirely possible mechanism for photoreactivation. In addition, chemical experiments show somewhat similar model systems. Nevertheless, many of the data on photoreactivation, especially the strong dependence on environment, indicate that this type of mechanism by itself is unlikely.

V. GENERAL REMARKS

One has the feeling that perhaps more is known of photoreactivation behavior than of ultraviolet behavior. There are some very basic questions that, in spite of the hundreds of ultraviolet experiments reported, still have not been satisfactorily answered. One of the most pressing is whether UV action is primarily direct or indirect. Satisfactory data for dose rate, temperature, and oxygen effects with UV are scarce. Few satisfactory absorption studies with microorganisms have been done in the range 2000 to 5000 A. Such work requires careful instrumentation; until it is done, quantum yields and efficiencies will not be known.

There are some grave experimental difficulties with UV that are not always recognized. In the bacteria, there is the perennial problem of strains B and B/r of *E. coli*. These strains are not biochemically defined. They are characterized only by their radiation resistance[15] and there may be great variation within either strain (there is some experimental evidence for this). Most UV restorations work on B much more effectively than on B/r, yet strain B shows atypical radiation response.

Bacterial systems (including phage and transforming factor) have been used extensively, largely because they lend themselves to quantitative procedures better than other organisms. It does not seem wise, however, to limit experimentation to these systems, for their structure and behavior may be quite specialized. For example, there seems to be no temperature coefficient for inactivation of bacteria with UV, but some paramecia show as high a coefficient as 3 for killing. An anomaly of the bacteria is their high concentration of nucleic acid. *E. coli* is about 14 per cent nucleic acid by dry weight. Considering its high specific absorption, it is not surprising that this substance should play a major role in UV effects. Other organisms, with a far lower concentration of nucleic acid, may be inactivated by UV in a different way. For example, bacteria are killed most efficiently and division of protozoa is delayed most efficiently by 2600 A, but killing of some protozoa shows a peak at 2800 A.

What may be expected from future work in photoreactivation? The most encouraging recent development is the discovery by Goodgal and coworkers of PR *in vitro*. It is expected that work with this system will soon elucidate much of the mechanism of PR in bacteria.

Two quite new techniques will probably be applied to the problem before long. One is paramagnetic resonance, which might indicate whether there is any immediate reversal of UV damage. The other is the UV and visible microbeam, which in some cases could resolve clearly the question of the location of chromophore and reactivable site.

VI. ACKNOWLEDGMENTS

The stimulus for writing this paper and some of the concepts developed herein grew out of informal discussions held in the Biology Division of the Oak Ridge National Laboratory on October 15 and 16, 1956. The meeting was attended by various members of the Biology Division as well as by Dr. G. H. Bowen (Iowa State College), Dr. R. Latarjet (Institut du Radium, Paris), and Dr. M. R. Zelle (Cornell University).

[15] Zimmerman (176), however, has recently shown a difference in colony morphology of cultures of B and B/r on a special medium. In future work, this new criterion of strain type should be applied whenever possible in order to determine whether it holds for all existing B/r cultures.

I wish to offer my sincere thanks to Drs. A. D. Conger, R. Dulbecco, A. Hollaender, A. Kelner, R. Latarjet, M. L. Randolph, C. S. Rupert, and G. E. Stapleton for their criticism of the manuscript.

ADDENDUM

Recently, Rupert (*personal communication*) has found that extracts of baker's yeast support PR of the transforming factor for streptomycin resistance of *H. influenzae* in a manner analogous to the action of *E. coli* extracts. However, only a *nondialyzable* component is essential for the photoreactivating power of these yeast extracts. Standard protein purification techniques have so far permitted a 20-fold purification of the active fraction, which is believed to be an enzyme. Competition for the action of this supposed enzyme is obtained by UV irradiated nontransforming *H. influenzae* DNA, and by UV irradiated phage T2 DNA. Unirradiated DNA and photoreactivated DNA do not appear to compete.

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